UNIVERSITY OF READING

Department of Agriculture

CHARACTERISATION OF UGANDAN ISOLATES OF *EXSEROHILUM TURCICUM* FROM MAIZE

by

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A thesis submitted in partial fulfilment of the requirements for the Degree of Master of Science in the Technology of Crop Protection.

September, 1992.

DEDICATION

Dedicated with thanks to my parents who instilled in me the great values that have guided me up to this point and for their sacrifices in educating me. Also to Dr. J.K. Ransom for his efforts in securing me the scholarship.

ACKNOWLEDGEMENTS

Special thanks go to my supervisor Dr. A.M. Julian for her guidance, patience and invaluable advice throughout my study period. All members in pathology section of Spur B especially Mr. A. Jama are acknowledged for their assistance in one way or another.

Many thanks to individuals who sent me materials to use in this project: Dr. C. De Leon for seeds of the isogenic differential lines, Mr. D. Baguma for isolates and seed, Dr. S. Gowen and Mr. T. Kalule for the seed.

I am very grateful to CIMMYT for offering me yet another scholarship. Dr. J.K. Ransom of CIMMYT East Africa regional office is greatly acknowledged for this.

Parents, relatives and friends are all thanked for their prayers, encouragement and moral support. Finally, I am indebted to my family: my wife Lydia, children Fred and Stella for their tolerance whilst I was away.

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ABSTRACT

Northern corn leaf blight (NCLB) is one of the major four diseases threatening the maize industry in Uganda, in addition to maize streak virus, common smut and rusts. This study characterised four representative isolates of *Exserohilum turcicum* obtained from the major maize growing districts in Uganda. Isolates were assessed with respect to their cultural variability, pathogenicity towards local maize lines and an isogenic series containing the Ht genes for resistance. Two other isolates one from Zimbabwe and a 2N isolates from USA were included for comparative purposes.

Experiments conducted *in vitro* indicated that radial growth rates between isolates were significantly different. Ugandan isolates tended to have higher temperature optima than the Zimbabwean, with the Kasese isolate growing the fastest. This correlates with typical temperature at the original location. The Zimbabwean isolate was obtained from Henderson Research Station with comparatively low temperatures between 8-26°C while Kasese district has a minimum temperature of 25°C.

Results obtained from the study of the effect of light regime on sporulation demonstrated that sporulation was favoured by continuous darkness while continuous light was inhibitory. The US (2N) isolate sporulated most vigorously.

Of the Ugandan isolates, the Masindi isolate produced the largest number of conidia, followed by Iganga, Mpigi and Kasese isolates.

Race typing of the Ugandan and Zimbabwean isolates was conducted in the greenhouse and under controlled environmental conditions in the growth cabinet. Results were more consistent under controlled conditions in the growth cabinet but still correlated well with those obtained in the greenhouse. The isogenic line (H4460) without the Ht gene developed classic necrotic susceptible type lesions following inoculation with all isolates whilst H4460Ht1, H4460Ht2 and H4460Ht3 exhibited the resistant chlorotic lesion type. Line A697(W22HtN) had a prolonged incubation period developing chlorotic flecks when inoculated with Ugandan and Zimbabwean isolates, but was susceptible to the race 2N from USA.

The relative susceptibility/resistance of six Ugandan maize cultivars (Babungo (3), Longe 1, topcross, singlecross, and population 28 and 29) was assessed using one Ugandan isolate (Kasese) and the 2N (US) isolate. Topcross, Longe 1 and Babungo (3) showed resistance to both isolates while population 28 and 29 were found to be highly susceptible. There was correlation between lesion length, percentage leaf tissue blighted (% LTB), lesion number, sporulation rating, sporulation and susceptibility. Susceptible cultivars had higher values for all parameters tested.

Reaction types varied, topcross did not develop any lesions when inoculated with the US isolate, whereas Longe 1 and Babungo (3) developed chlorotic flecks and population 28 and 29 developed chlorotic type lesions but had longer incubation periods compared with the Kasese isolate.

CHAPTER 1

GENERAL INTRODUCTION

1.1 The crop

Maize, *Zea mays (L.)* belongs to the family Graminae, tribe Maydeae which includes genera of both new and old World origin. One of the New World genera is *Zea* which includes *Zea mays* and *Zea mexicana* also classified as subspecies *mexicana* of *Zea mays* (Goodman, 1976; Galinat, 1977). This species was originally classified as a separate genus as *Euchlaena mericana*.

Maize is diploid (2n=20), and is able to outcross with teosinte (*Zea mexicana*) to give fertile progeny (Mangelsdorf, 1974). Races within *Zea mays* were first categorised in the 19th century solely on endosperm characteristics. However, subsequent classification has been based on endosperm characters (Brown and Goodman, 1977) which still form part of the primary description of races:

1. Pop maize (*Zea mays everta*) : small smooth kernels with hard endosperm
2. Flint maize (*Z. mays indenturata*) : large smooth kernels mainly hard endosperm but often with a small floury centre
3. Floury maize (*Z. mays amalacea*) : large smooth kernels with floury endosperm
4. Dent maize (*Z. mays indenta*) : large kernels with a

central core of floury endosperm which, on drying, shrinks more than the surrounding hard tissue denting the kernel; it is the leading type by volume of world production

5. Sweet maize (*Z. mays succharata*) : large kernels wrinkled and translucent when dry

6. Pod maize (*Z. mays tunicata*)

7. Waxy maize (*Z. mays ceratina*).

1.1.1 Systematics and history of development

The origin of maize remains uncertain and has been the source of much controversy (Mangelsdorf, 1974; Goodman, 1976; Randolph, 1976; Galinat, 1977; Heiser, 1979). Galinat (1977) summarises the three current tenable hypotheses as follows:

1. The current Teosinte is the common ancestor of maize

2. A primitive form of teosinte is the common wild ancestor of both maize and the current teosinte

3. An extinct form of primitive maize was the ancestor of maize, teosinte being a mutant form of the primitive type.

The earliest archaeological evidence for the geographic origin of maize is from caves in the Tehuacan Valley, Mexico dated about 7000 BP (Mangelsdorf, MacNeish and Galinat, 1967) but whether the cobs found are those of wild maize (Mangelsdorf, 1974) or and early domestication (Galinat, 1977) is not currently known. Subsequent dissemination throughout the world was probably by Portuguese navigators of the sixteenth and seventeenth

centuries (Rouanet, 1987).

Maize was introduce into Africa by two routes. The Portuguese introduced maize derived from Central and South to the West African coast in the early sixteenth century (Miracle, 1965), however, linguistic evidence suggests that many areas of tropical Africa also received maize from the Mediterranean region via the Sahara. Maize was established as a primary crop in the interior of W. Africa by the later eighteenth century and in the Congo Basin after 1930. Maize was unknown in Uganda until after 1861 (Normal *et al.*, 1984; Kaahwa *et al.*, 1985); and did not become established as a staple until the twentieth century.

1.1.2 World importance and tonnage

Maize is generally grown between latitudes 30° and 47° (Shaw, 1977). Approximately 450 million tons are produced world-wide each year making this crop second to wheat as a cereal crop of world wide importance (Hill and Sykes, 1975). The main reason for these high production figures derives from two principal features; firstly the status of maize as a high energy source per unit consumed and secondly, its versatility for both grain and forage production. Although the tonnage of world wheat production exceeds that of grain maize by one seventh, in energy terms the gap is only of the order of six to seven percent. The United States produced nearly half the world's maize in 1982 (see table 1) whereas Africa accounted for only 3.5%

(Rounet, 1987). Brazil, Mexico and India are the only three of the thirteen developing countries producing more than 5 metric tons a year (Norman *et al.*, 1984).

Table 1: Major maize-producing countries: area, yield and production (Averages 1978-80)

Country	Area (1000ha)	Yield Kg ha^{-1}	Production (1000t)
USA	29 325	6 310	185 041
China	19 745	2 919	57 637
Brazil	11 292	1 484	16 752
Romania	3 263	3 454	11 271
Mexico	6 647	1 511	10 045
France	1 853	5 212	9 657
South Africa	5 667	1 670	9 467
USSR	2 901	3 105	9 008
Yugoslavia	2 183	4 088	8 925
Argentina	2 624	3 126	8 203
Hungary	1 338	5 244	7 017
Italy	930	6 801	6 325
India	5 771	1 050	6 059

After FAO (1981).

1.2 Maize production in Uganda

Uganda's principal food crops are plantains (matooke), sweet potatoes, cassava, maize, finger millet, sorghum, beans, groundnuts and simsim. In 1984, maize became Uganda's third largest source of foreign exchange, when 40,000 metric tons were exported for US$ 10 million (Uganda Ministry of Planning and Economic Development reports, 1987-1990). Since the 1980s, maize hectarage and yields per hectare have continued to increase (see table 2) (FAO year book 1987-1990) and in 1989 production reached 1.2 million tons.

Table 2: Maize Production in Uganda.

Year	Area ('000)	Yield (Kg/ha)
1979-81	263	1 361
1985	271	930
1986	282	887
1987	280	1 182
1988	345	1 275
1989	360	1 389
1990	380	1 447

(FAO Yearbook Production, 1987-90)

1.2.1 Geographical distribution

Maize is grown throughout Uganda. In urban areas where there is high demand for green maize it is cropped throughout the year in swampy areas, elsewhere there are two plantings. The major maize growing districts in Uganda include: Kasese, Masindi, Iganga, Masaka (Uganda Ministry of Agriculture, Statistics unit, 1900) (Figure 1).

1.2.2 Farming types

Agriculture is the most important sector of Uganda's economy accounting for about 95% of the country's export earnings, 55% of gross domestic product (GDP) and providing a livelihood for 82% of Uganda's labour force (Uganda Ministry of Agriculture reports 1991/92; Ministry of Planning and Economic Development reports 1989-90). There are three categories of farmers: small scale (subsistence), small holders and large scale farmers who plant an average of 1.5 ha of maize either as a mono crop or intercropped mainly with beans. Few subsistence farmers have access to tractors for land preparation or inputs such fertilizers and pesticides. They grow mixtures of varieties - improved and their own selection (Okoth *et al.*, 1990). Small holder farmers constitute about 32% of the population. These farmers tend to grow maize as a commercial crop, buying improved seed from the national seed project and can afford some inputs and machinery hire or use on their fields. The commercial farming sector comprises about 18% and most own their own agricultural machinery, use improved seed and can

afford inputs such as herbicides, fertilizers and insecticides (Okoth *et al.*, 1990). Some small scale farmers in Eastern Uganda used own oxen for traction of farms but now because of cattle rustling animal traction is no longer in use.

1.2.3 Constraints to maize production

1.2.3.1 Drought

Although maize is cropped twice a year in Uganda (March-April and August-September) it is not always possible to produce two harvests in some regions. In the western parts of the country (Kasese, Mbarara, Rukungiri and Bundibogyo) the August-September rains are generally longer than the March-April rains. In the east and north (Iganga, Tororo, Mbale, Lira Gulu and Apadi) the March-April rains are longer than August-September. However, in some years eg. 1988 and 1991 the long rains are erratic and maize production is severely affected.

1.2.3.2 Declining fertility

Many soils in Uganda are nutritionally poor, having lost fertility either as a result of overcultivation or leaching. The majority of subsistence farmers are unable to afford fertilizers and others continuously crop in the same field each season resulting in soil exhaustion and therefore low yields.

1.2.3.3 Marketing system

The distribution and marketing system for both food and cash crops is generally poor in Uganda. Marketing is usually mediated by the government through parastatals such as the Produce Marketing Board (PMB). The government is slow to raise the finances to buy the produce from the farmers. Farmers are therefore forced to store their produce for long periods resulting in spoilage due to weevils, rodents and storage fungi. In some cases the middlemen profit from the delay by offering lower prices to the farmers.

1.2.3.4 Pests and diseases

The stem borers: *Chilo partellus, Busseola fusca, Eldana saccharina* and *Sesamia calamistis* are the major insect pests of maize in the field. Grain weevils (*Sitophilus spp*) cause serious yield losses to maize in storage (Okoth *et al.*, 1990; Uganda Maize Research Programme annual reports 1988-91). The parasitic weed *Striga hermonthica* is also posing a big threat to farmers in some of the major maize growing districts for example, Kasese, Masindi, Tororo, Lira, Soroti and Apac (Okoth *et al.*, 1990).

The most important maize disease in Uganda include northern corn leaf blight (NCLB) caused by *Exsesohilum turcicum*; maize streak virus, tropical and southern rusts (*Puccinia sorghi* and *P. polysora* respectively); common smut *Ustilago maydis* and ear rots (*Fusarium monilliforme* and *Diplodia*

maydis) (Bigirwa, 1990; Maize Programme annual reports 1988-91). A survey carried out in Uganda by Okoth *et al.*, (1990) in the 1990 growing seasons indicated that NCLB, maize streak virus and rusts were the predominant diseases in the districts surveyed. The incidence of the three was calculated at 57.4%, 10.5% and 24.6% respectively (Okoth *et al.*, 1990).

1.3 Current strategies for improvement (breeding programmes)

1.3.1 CIMMYT breeding programme

NCLB is one of the priority diseases addressed by CIMMYT in its maize improvement programmes both at headquarters in Mexico and at the sub-regional centres (CIMMYT, 1982). Various strategies are used to screen and breed for NCLB resistance including mass selection, recurrent selection and back crossing (Ceballos *et al.*, 1991). One of the populations developed by CIMMYT known to be NCLB resistant is population 42-ETO-Illinois (CIMMYT, 1988). Other breeding programmes are geared towards developing open pollinated varieties and hybrids resistant to maize streak virus, rusts, ear rots and stalk rots. CIMMYT also breeds varieties to suit various ecological zones worldwide.

1.3.2 Uganda national maize research programme

Following a severe outbreak of NCLB in Uganda in 1988 this pathogen became one of the more intensively studied

diseases in the country (Kyetere and Bigirwa, 1989). A range of germplasm has been screened and categorised; and sources of resistance identified and incorporated into new lines (Maize research programme annual reports 1988-90). In addition, other diseases addressed include : common smut, ear rots, brown spot, maize streak virus and rusts.

1.4 The disease/pathogen

1.4.1 Economic importance of NCLB

NCLB is widespread in many regions where maize and sorghum are grown (Tarr, 1962; Shurtleff, 1980). Disease severity depends on favourable environmental conditions, inoculum density and host susceptibility (Levy, 1991). In general non-systemic foliage diseases do not kill the plant, except where seedlings or young plants are exposed to prolonged severe attack (Tarr, 1962). The major effects of NCLB are caused by destruction of photosynthetic tissue and in some instances premature withering and leaf shedding leading to reduction in plant growth (Tarr, 1962). NCLB is regarded as the most important leaf disease of dent maize with severe attacks reducing grain yields up to 50% (Ullstrup, 1977). Maize is most susceptible to NCLB 2-3 weeks after pollination with epidemics occurring 6-8 weeks after pollination giving no significant yield losses (Ullstrup and Miles, 1957). It has been estimated that 0.23% yield index was lost for each 1% NCLB severity 3-4 weeks after mid-silk (Fisher *et al.*, 1976). It has also been shown that

yield loss due to NCLB could be correlated with the area under the disease progress curve (AUDPC) in a year with high disease severity, but not in a year with low severity (Raymonds and Hooker, 1981).

1.4.2 Systematics

Symptoms of NCLB were first described on maize in Italy by Passerini in 1876 (Tarr, 1962). NCLB is caused by the fungal pathogen *Exserohilum turcicum* (Pass.) Leonard and Suggs (syns. *Helminthosporium turcicum* Pass; *Bipolaris turcica* (Pass.) Shoem., and *Drechslera turcica* (Pass.) Subram. and Jain). Its perfect state is *Trichometasphaeria turcica* Luttrell (syns. *Setosphaeria turcica* (Luttrell) Leonard and Suggs) (Bergquist, 1986).

There is currently some confusion as to the correct nomenclature for this pathogen, as the generic name *Helminthosporium turcicum* is still widely being used. The genus *Helminthosporium* was first segregated into sub-genera *Cylindro - Helminthosporium*, based on conidial morphology and type of conidial germination (Nasikado, 1928 and 1929). Ito (1930) raised the sub-genus *Cylindo - Helminthosporium* into generic rank as *Drechslera*. Shoemaker (1959) established the genus *Bipolaris* for a species that Nasikado (1928) had placed in sub-genus *Eu-Helminthosporium*. The former segregated genera is often associated with *Pyrenophora* and the latter with *Cochliobolus* telemorphs (Sivanesan, 1987 and Alcorn, 1988b).

The basis for separation of *Exserohilum* from *Bipolaris*, *Drechslera* and *Helminthosporium* is based the conidial hilum which is distinctly protuberant (Alcorn, 1988b). Other characters used for separating *Exserohilum* from other genera include: the point of origin of germ tubes and direction of its growth from the basal cell of conidium, the ontogeny of the conidial septum, the hilum morphology, the delimitation of the basal cell and the type of conidiogenous node surface and also the conidial shape, colour and curvature (Alcorn, 1983, 1986, 1988a and 1988b). The differentiating criteria for categorising *Bipolaris, Drechslera* and *Exserohilum* (as described by Alcorn, 1988b) is summarised in Table 3.

Table 3: Differentiating criteria for Bipolaris, Drechslera and Exserohilum (from Alcorn, 1988b)

Criteria	Bipolaris	Drechslera	Exserohilum
1. Hilum morphology	Often slightly protruding, truncate and the hilum is characterized by a flattering of the contour of the basal cell	Non-protruding, hilar region of the basal cell is smoothly rounded and having well-defined intra-hilar cavity	Strong protruding truncate and the protrusion is clearly double walled for all or part of its length with an outer wall forming an enveloping collar or 'bubble' around it
2. Basal cell germination: position and direction of basal germ tube (Fig. 2)	Usually emerges immediately adjacent to the hilum and grows in the direction of the long axis (semiaxially) rarely lateral	Emerges in a median position between the hilum and the septum; rarely semiaxial from near hilum	Similar to Bipolaris
3. Germination: number and position of cells that germinate	Commonly from one or both polar cells, rarely from intermediate cells	Any cells: from intermediate and/or polar cells	Similar to Bipolaris
4. Septum development (Fig. 2)	Primary septum is median to distinctly submedian; second septum delimits the basal cell and the third towards the apex i.e. distal. Some variability has been reported (Alcorn, 1983 and Sivanesan, 1985).	First septum delimits the basal cell; second one is median and third is distal.	First septum is submedian; the second is distal and cuts off an apical cell (supra-median) and the third is median. The position of second and third septa is more variable than other two genera (Alcorn, 1988b).
5. Conidial shape	Fusoid, obclavate fusoid, rarely truly cylindrical; straight or curved.	Commonly cylindrical or obclavate cylindrical; mostly straight	Fusoid, obclavate-fusoid or cylindrical; straight or curved
6. Conidial colour	Darker	Pale	-
7. Conidiogenous loci on the conidiophore	Commonly roughened	Usually smooth	As in Bipolaris

1.4.3 Morphology

1.4.3.1 Conidium

Exserohilum turcicum produces broad ellipsoid or obelavate ellipsoid, spindle shaped, slightly curved conidia, borne singly at the tips of the conidiophores (Tarr, 1962; Luttrell, 1964; Shurttlef, 1980; Alcorn, 1988b). The mature conidia are widest near the middle and taper towards both ends to give a fusoid shape. Both ends are reduced but the basal portion of the conidium has a distinct hilium (Tarr, 1962). Young conidia are sub-hyaline becoming brownish-yellow to olive-gray when mature. Most are between 73-137 x 18-23 μm with 4-9 cross septa although up to 11 may be present (Tarr, 1962; Luttrell, 1964; Shurtleff, 1980).

1.4.3.2 Conidiophore

The conidiophore are olivaceous in colour, 6-10 μm in length; they are often 2-4 septate (Tarr, 1962; Luttrell, 1964; Shurtleff, 1980). The hyphal multinucleate with all nuclei in a cell dividing simultaneously, the conidiophores subsequently emerging in groups of 2-6 or more (Knox-Davies, 1959).

1.4.4 Cultural growth

1.4.4.1 Media for growth and sporulation

E. turcicum can be grown on a variety of media including cornmeal, barley, rice and sorghum meal agars (Mitra, 1923); rice decoction agar (Nisikado, 1923); Richard's medium; Czapek's medium; glucose peptone; potato dextrose;

and maize leaf extract media (Singh, 1958; Misra and Roy, 1965).

Sporulation can be induced on Hopkin's nutrient solution (solidified with 2% agar) (Nisikado, 1927); glucose peptone and Richard's media (Misra and Roy, 1965). Lactose casein Hydrolysate agar (LCH) is cited as inducing high levels of sporulation at pH 6 (Tuite, 1969).

The optimal temperature for the growth of *E. turcicum* in culture has been described as 27-30°C, with a minimum of 8°C and a maximum of 35°C (Luttrell, 1964; Tuite, 1969; Alcorn, 1988). Sporulation *in vitro* is also influenced by temperature, 28°C being the optimum. When cultured on LCH abundant sporulation can be attained at 24°C (Bergquist and Masias, 1988). Conidiation is inhibited at 28°C under a continuous light regime, although growth in continuous light at 28° for 7 days, followed by exposure to darkness at 24°C for 24 hours results in abundant conidiation (Bergquist and Masias, 1988).

1.4.4.2 Factors affecting size of conidia

Conidial size, length, breadth and septation can be variable and are influenced by both environmental and genotypic factors (Drechslera, 1923; Tarr, 1962; Alcorn, 1988). The morphology of a single spore isolate can be influence by culture media, temperature, light intensity, pH and age (Elliot, 1949; Misra and Roy, 1965; Kafi and

Tarr, 1966; Tarr and Kafi, 1968; Ruppel, 1974; Harding, 1975; Dalmaico, 1986).

1.4.5 Symptoms and effects on host

Symptoms of NCLB are long elliptical grayish-green or tan lesions with initial disease symptoms first appearing on the lower leaves of the plant (Ullstrup, 1977; Levy, 1984). Within 48 to 72 hour period after infection of the leaves by conidia, small chlorotic flecks appear at penetration points. Lesions first become clearly visible 8 to 10 days after infection (Ullstrup, 1977). The time required for full lesion development depends on temperature. At lower temperatures a longer time is required.

It is not always possible to identify the causal agent of NCLB by symptoms alone, as two or more *Helminthosporia* may attack the same leaf giving similar symptoms (Robles, 1949, Aldrich *et al.*, 1975). Leaf blights causing symptoms similar to those of NCLB are described in Table 4. Additionally, under some conditions saprophytic fungi may modify the type of lesion produced (Robles,1949; Levy,1984).

Table 4: Comparison of Appearance of Leaf Blights

An expert may be needed for positive identification but there are differences which can be learned with careful study and a little field experience.

	Northern *Helminthosporium turcicum*	Southern *Helminthosporium maydis* Race O	Race T	Yellow *Phyllosticta maydis*	Eyespot *Kabatiella zeae*	Brownspot *Physoderma maydis*
Growth stage when symptoms appear	Silking or later	First cycle may show on plants when about 24in (60 cm)	Seedling from infected seed may die within 3-4 weeks. Leaf symptoms same time as Race O.	Before tasseling	On small plants	Young plants infected in leaf whorl.
Part of plant affected	Lower to middle leaves first. May spread to	Usually only leaves.	Leaves, stalks, leaf sheaths, husks, shanks, ear.	lower leaves, especially outer margins. Also leaf sheaths and husks.	Mainly upper leaves of plants nearing maturity. Some on husks and leaf shaths	Leaf, sheath, stalk and sometimes husks and tassels. Bands of infection on leaves is common.
Color of lesions	Gray, frosted, especially lower two thirds of plants.	Premature dying of entire plant. More tan than gray.	Same as Race O. Close inspection reveals infection of all parts except tassel.	Yellow lower leaves resemble nitrogen deficiency. Follwed by death. More		Brown to reddish brown, much stalk break.
Size of legions						
Width	½-3/4 in. (1.25-1.6cm)	¼-½ in. (.6 to 1.25cm)	Same as O.	⅛in. (.3cm)	1/10-6/10 in. (1-4mm) in diameter surounded later by a dark ring and outer halo	Very small early but coalece into large patches.
Length	1-6 in. (2.5-15cm)	½-3/4 in. (1.25-1.6cm)	1-1.5 2.5-3.75cm)	½in. (1.25cm) plus halo		
Shape of lesions	Elongated, elliptical	Parallel sides	Elliptical with yellow-green or chlorotic halo	Rectangular to oval sorrounded by yellow, chlorotic tissue.	Circular to oval with dark ring and outside halo resembling an eye	Oblong to round. round.
Appearance of field in late stages of severe infection	Tan turning more gray with age	Tan with buff to brown borders	Tan with yellow-green to chlorotic halo later turning dark, reddish brown	Yellow, cream or tan often surrounded by chlorotic tissue. May coalesce and cause leaf tissue, especially margins to die.		Yellowish early, brown to reddish brown later
Geographic distribution	Most humid areas of the world.	World wide, especially warm, damp climate.	More northern areas than Race O.	Widespread but most troublesome in northern corn Belt.	Northern U.S., Canada, Japan and perhaps Brazil, Australia, Argentina.	Mainly localized in S.E. United States but sometimes in midwest.

1.4.6 Life cycle

1.4.6.1 Host-pathogen interaction

Epidemics result from the interaction between a virulent pathogen with a susceptible host in a favourable environment through time (Fry, 1982; Agrios, 1988). Where conditions are unfavourable for development, pathogens often evolve mechanisms by which they can survive until the situation improves e.g. overwintering as mycelium in infected tissues (Agrios, 1988). *E. turcicum* is able to survive for at least one year as dormant mycelia and spores in dried leaves although spores can lose their viability with time (Ullstrup, 1977).

E. turcicum may also exist on collateral hosts eg wild sorghum species and volunteer maize at the field edge (Tarr, 1962; Shang, 1980; Levy, 1984; Bergquist, 1986). Boosalis *et al.*, (1967) demonstrated that conidia overwintering on maize residue were able to provide the initial inoculum source. Initial inoculum may also be provided by chlamydospores carried by maize residue or by conidia produced on *Sorghum halepense (L.)* Pers., a perennial host of the pathogen (Levy, 1984). The appearance of the initial symptoms on the lower leaves of the plant may also imply that the soil/debris act as a source of primary inoculum. Host genotype and pathogen race may also influence the survival of the overwintering pathogen (Nelson and Scheifele, 1970). In addition to crop debris, the sudden appearance of localised epidemics in fields with

no appreciable amount of crop residue, may be attributed to aerial inoculum from 'spore showers' (Hope and Arny, 1966).

1.4.6.2 Environmental requirement for disease development

A combination of moderate temperatures (20-25°C) and high relative humidity (90-100%) are considered essential for disease development (Leach *et al.*, 1977; Levy and Cohen, 1983), and dry weather retards disease development (Bergquist, 1986). However, in practice, the incidence of NCLB can be low when environmental conditions are optimal and severe epidemics sometimes occur under sub-optimal environmental conditions (Levy, 1989). This may be due to the presence of a highly aggressive population of the pathogen, or a highly susceptible host.

1.4.6.3 Sporulation on leaf tissue

Sporulation requires continuous periods of high atmospheric humidity (RH>90%) probably occurring only at night (Leach *et al.*, 1977). The conidia flick off the 'hilum' and are dispersed by air currents when the conidiophore snaps to an upright position. This process often takes place in the mornings as the sun dries the leaf surface (Meridith, 1965; Frederiksen, 1978). Diurnal changes resulting in decreases and increases in relative humidity, and the 'trigger mechanism' of spore release under natural conditions result in a bimodal pattern of release. One peak occurs during the morning as the relative humidity decreases, and the other peak in early evenings as the relative humidity increases

(Leach, 1975).

1.4.6.4 Spore landing and development in host

NCLB is a polycyclic pathogen and secondary spread within and between fields is due to conidia dispersed by air currents (Bergquist, 1986). For infection to occur free water must be present within 6-18 hours at 18-27°C (Holliday, 1980). Following attachment conidia germinate to form a germ tube which subsequently forms appressoria on the leaf surface. An infection peg then develops under the appressorium and penetrates through the cuticle subsequently forming hyphae within host cells (Frederiksen, 1978). The majority of penetration events result in the appearance of a hypersensitive fleck indicating an incompatible reaction (Tuleen and Frederiksen, 1977; Ullstrup, 1978). Successful hyphae grow through living cells forming rudimentary appressoria on each cell wall before continuing. Hyphae growing adjacent to vascular bundles, enter a xylem vessel and proliferate (Jennings and Ullstrup, 1957), causing damage as the mycelium plug the vessel and restrict the transpiration flow (Frederiksen, 1978). Following lesion development sporulation occurs on the leaf surface. Hyphae fill the substomatal cavity or epidermal cell and produce a stroma from which conidiophores of *E. turcicum* develop.

1.5 Control

1.5.1 Cultural

Epidemiology data imply that the major inoculum source for NCLB epidemics is infected crop residues (Robert and Findley, 1952). Fungal material within diseased maize leaves can persist throughout the dry season developing conidia when exposed to favourable conditions the following season. It has been observed that conidia remaining on the surface of the lesions were unable to germinate after drying at 40°C, however, mycelia were able to survive in dried maize leaves up to a year (Tarr, 1962).

1.5.1.1 Sanitation

Inoculum sources could be reduced by crop sanitation measures including the destruction of crop residues, stubble, alternative host plants and volunteers (Tarr, 1962; Frederiksen, 1986; Bergquist, 1986). Destruction could be by burning, manual removal, feeding to livestock or deep ploughing.

1.5.1.2 Change in planting date/germplasm distribution

It is possible to reduce initial infection by altering the planting date to avoid the warm wet climatic conditions which favour the spread of the disease or only growing blight susceptible cultivars in drier areas. These practices are however, limited for irrigated crops as the high levels of moisture required for optimum growth of maize is often sufficient for development and spread of

NCLB (Tarr, 1962).

1.5.2 Mineral levels

A link has been established between NLCB severity and availability of potassium, as KCl such that NCLB is less severe when the supply of potassium is adequate (Hooker *et al.*, 1963). The relationship between potassium level and disease severity is most pronounced on impoverished soils because the plant is physiologically stressed and therefore more susceptible. However, environmental conditions and host resistance are the most important factors in determining the severity of this disease rather than host nutrition (Ullstrup, 1977).

1.5.3 Fungicide usage

A range of chemicals applied both as seed dressings and foliar applications have been screened. Foliar applications of fungicides are most appropriate for seed production fields to be applied when lesions are first observed, as done by some farmers in Zimbabwe (Julian personal communication). However, foliar applications are not economical for small holder or subsistence farms (Bergquist, 1986). The protective fungicides Maneb and Zineb give satisfactory control (Molot and Simone, 1961). Manzate 200 (Maneb) at 900 g/ha or Dithane M-45 F1 at 0.54 g/ha reduced the percentage of the leaf area blighted by *E. turcicum* and *Bipolaris maydis* (White, 1981a,b). Dithane Z-78 at 300 mg/100 ml of water and Dithane M-45 at 143 mg/100

ml of water were also shown to control *E. turcicum* (Power and Patil, 1978).

1.5.4 Breeding for resistance

The development of varieties with resistance to diseases offers the most practical long term means of control (Hilu and Hooker, 1964; Russel, 1978; Fry, 1982). The type of resistance can vary ranging from small effects where lesion size is limited or latent period extended to complete suppression. Even forms of resistances that do not wholly suppress pathogenesis may reduce disease levels such that yield losses are negligible (Fry, 1982).

1.5.4.1 Monogenic resistance

Genetic resistance in maize to infection by *E. turcicum* can be monogenic or polygenic (Hooker, 1961b; 1963). Monogenic (=vertical, race specific) resistance is usually controlled by one or a few major genes eg. the Ht genes (Russel, 1978; Argios, 1988). This form of resistance is effective against specific races of the pathogen, and ineffective against others. It operates on the Gene for Gene concept (Vanderplank, 1982; Argios, 1988). Monogenic resistance is not desirable as a result of selection for new pathogen races able to by-pass or overcome it (Argios, 1988).

The expression of Ht resistance in maize is commonly referred to as chlorotic (fleck) lesion resistance giving non-sporulating chlorotic lesions (Hilu and Hooker, 1946;

Ullstrup, 1970; Kinsey and Hooker, 1974). It is controlled by a single dominant gene derived from inbred-line GE 440 and Ladyfinger popcorn (Hooker, 1961). Three other genes Ht1, Ht2 and Ht3 were identified later (Hooker and Perkins, 1980).

Ht1 was derived from inbred GE 440 and ladyfinger popcorn
Ht2 was derived from the Australian dent maize inbred NN14B
Ht3 was derived from *Tripsacum floridanum* to maize through interspecific hybridization and chromosomal translocations (Hooker, 1963).

A further resistance gene HtN was later derived from the Mexican corn variety Parlevliet in Zimbabwe (Parlevliet, 1979; Leath and Pedersen, 1983). The monogenic Ht resistance is dominant and is therefore suited to rapid screening techniques (Hooker, 1977) and is also relatively simple to transfer from one source to another. However, the chlorosis associated with Ht1 and Ht2 can be detrimental to the plant under severe epiphytotics of NCLB (Ullstrup, 1970). In addition rapid development of new races may shorten the durability of these sources of resistance (Ceballos *et al.*, 1991). Ht1, Ht2 and Ht3 genes control rate of lesion expansion and sporulation. HtN also increases incubation time and latent period (Hooker, 1977; Leath and Pedersen, 1983; Pataky *et al.*, 1986). Ht1 and Ht2 are not linked and interact to condition higher levels of resistance (Hooker, 1977).

1.5.4.2 Monogenic resistance associated with phytoalexins

The resistance conferred by the Ht genes may be mediated by production of anti-fungal compounds such as phytoalexins and hydroxamic acid (Lim *et al.*, 1970; Couture *et al.*, 1971). Resistance to infection and subsequent invasion of host tissue by *E. turcicum* in genotype bearing the gene Ht has been correlated with two phytoalexins A1 and A2 (Ullstrup, 1977). The compounds are not detected in healthy tissue but are elicited by the host in response to general wounding such as that caused by penetration of *E. turcicum* appressoria. When a drop of spore suspension is placed on a leaf, phytoalexins are detectable in the droplet (diffusate) after 3 days. Isolates of *E. turcicum* from johnson grass, which are avirulent on maize did not elicit production of phytoalexin in infection droplets placed on maize leaves. Virulent isolates of *E. turcicum* derived from maize excited higher concentrations of phytoalexins than those of lesser virulence (Lim *et al.*, 1968).

Cyclic hydrosamate (hydroxamic acid) 2,4-dehydroxy-7-methoxy-1, 4 benzoxazinc 3-one (DIMBOA) does not exist constitutively, but is elicited in response to penetration, or injury, as a result of activation of a glycoside precursor. The latter compound inhibits spore germination but otherwise exhibits low levels of fungi-toxicity (Long *et al.*, 1975; 1978; Ullstrup, 1977). Dimboa produced by other plant species has a inhibitory effect on insects, fungi and bacteria and is commonly involved in resistance

to these organisms.

1.5.4.3 Polygenic resistance

Polygenic resistance is expressed in some genotypes (Jenkins and Robert, 1952; 1959; 1961; Hughes and Hooker, 1971). Polygenic (=multigenic, horizontal, non-race specific, rate reducing, partial) resistance is controlled by many genes (Russel, 1978; Agrios, 1988). These genes appear to operate by controlling the physiological processes involved in defense mechanisms of the plant. This form of resistance does not protect plants from becoming infected, but it slows down the development or spread of the disease (Agrios, 1988).

Polygenic resistance was the first form of resistance to be exploited for control of NCLB in maize (Ullstrup, 1977). It is determined by quantitative characters and is manifested in three ways: by reducing the number of NCLB lesions formed; extending the time for sporulation by a newly formed lesion; and decreasing the number of spores formed in lesions on a resistant variety (Russel, 1978; Brewster *et al.*, 1992).

1.6 Race structure

Although pathogenic specialization in *E. turcicum* conforming with the gene for gene concept had been reported in the early 1960s (Robert, 1960; Nelson *et al.*, 1965),

distinct race designations were not assigned to virulence types until 1972, when Bergquist and Masias (Bergquist and Masias, 1974) found isolates of *E. turcicum* virulent on inbred corn lines with Ht1. Two systems for race designation of *E. turcicum* have been described. These are summarised in Table 5. In the old system, 4 races were identified: (1: Ht1,Ht2,Ht3/0; 2: Ht2,Ht3/Ht; 3: Ht/Ht2,Ht3; 4: Ht1/Ht2,Ht3,HtN). However, this system was improved in 1989 by Leonard *et al.*, to give 5 races (0: Ht1,Ht2,Ht3,HtN; 1: Ht2,Ht3,HtN/Ht1; 23: Ht1,HtN/Ht2,Ht3; 23N: Ht1/Ht2,Ht3,HtN; 2N: Ht1,Ht3/Ht2,HtN). In this study the new system is referred to.

Race 0 is widely distributed in many parts of the world (Abadi *et al.*, 1989). Race 1 was first detected in 1974 in Hawaii (Bergquist and Masias, 1974) but is now widespread throughout the USA corn growing regions (Leath *et al.*, 1987). Race 23, appears restricted to the continent of America (Pedersen and Brandenburg, 1986). Race 2N was first reported in 1991 in Hawaii but has subsequently been observed in Texas (Thakur *et al.*, 1989).

To obtain the most reliable results, race identifications of isolates of *E. turcicum* should be based on the disease reactions of plants grown in controlled environment chambers: temperature 22/18°C day/night; and light intensities between 25 and 50 klux (about 325-650 $\mu Em^{-2}s^{-1}$ average photosynthetic photon flux density) (Leonard *et*

al., 1989). Host resistance has been shown to break down at low light intensity, and virulence reduced at high temperatures (Leath *et al.*, 1987; Thakur *et al.*, 1989).

Table 5: Pathogenic race nomeclature for *E. turcicum*

New race designation	Old race designation	Disease reaction Ht1	Ht2	Ht3	HtN
O	1	R	R	R	R
1	2	S	R	R	R
2[a]	----	R	S	R	R
12[a]	----	S	S	R	R
23	3	R	S	S	R
23N	4	R	S	S	S

R = resistant, S = Susceptible.

[a] Hypothetical races not yet found (Leonard *et al.*, 1989).

1.7 Objectives

Quantitative information on the prevailing races of NCLB in Africa (except Egypt), level and source of resistance and disease yield relationships for this region are limited. The race composition of *E. turcicum* in Uganda is unknown, however, this information is extremely important for designing breeding strategies for introducing genes for disease control.

The level and type of reaction of some Ugandan maize cultivars to *E. turcicum* has not been described to date. In this study the most recently released maize variety, Longe 1 and two new hybrids scheduled for release in the near future were assessed with other varieties. Differences in resistance expression by maize genotypes to *E. turcicum* are evaluated by measuring differences in different components of resistance for example : incubation period, sporulation ability, lesion expansion rates and apparent infection rates (Vanderplank, 1963; Raymundo and Hooker, 1982).

To up-date the information available on the etiology of *E. turcicum* in Uganda, this study was designed to address the following objectives:

(i) characterise the races present in Uganda;

(ii) describe the growth characteristics *in vitro* and *in vivo* of isolates from different parts of Uganda;

(iii) determine the susceptibility of Ugandan open

pollinated and hybrid maize cultivars to *E. turcicum* from Uganda and elsewhere. It is hoped that the findings will provide fundamental information for the national maize research programme in Uganda.

CHAPTER 2

EXPERIMENTS

2.1 Pathogenicity tests

2.1.1 Experiment 1

Characterisation of races of *E. turcicum* present in Uganda

Materials and Methods

Pathogen isolates: Four isolates of *E. turcicum* were obtained from the major maize growing districts in Uganda: Kasese, Masindi, Mpigi and Iganga (figure 1). Additionally two isolates from different geographic locations were included for comparative purposes. A Zimbabwean isolate from Henderson Research Station in Zimbabwe was chosen because of the international germplasm exchange programme through CIMMYT. The USA (2N) isolate from Hawaii was included as a known standard containing the Ht2N gene for resistance!. The US isolate was a gift from J. Windes, University of Illinois.

Preparation of single-spore cultures: Lesions of NCLB were excised, surface sterilised in 1% NaOCl for 1 minute, rinsed in sterile distilled water (SDW) for 3 minutes and transferred to moist filter paper in glass petri dishes. Cultures were incubated for 3 days at room temperature (25°C) to induce sporulation. Single conidia were transferred asceptically on to plates containing LCH (see

appendix 1 for recipe), and incubated at 25°C. After 10 days, mycelial plugs from edge of colony were transferred on to LCH to induce mass sporulation. Isolates were incubated at 25°C with a 12 hour light/dark regime. Conidia suspensions were prepared by washing 14 day old cultures with SDW and filtering the suspension through muslin cloth. Spore concentrations were then adjusted to $4x10^4$ spores/ml using a haemocytometer.

Host Plants: Five isogenic differential lines containing Ht resistance genes (H4460, H4460Ht1, H4460Ht2, H4460Ht3) and A697(W22HtN) were kindly provided by Dr. C. De Leon of CIMMYT Asian Regional Maize Programme. Three seeds were sown in 13 cm diameter plastic pots containing peat based multipurpose compost. For each treatment (isolate x host) 5 replicate pots were sown. Following emergence, seedlings were thinned to two per pot. Plants were grown in a heated glass house with no additional lighting. Thirty day old plants were inoculated by pipetting 1 ml ($4x10^4$ spores/ml) spore suspension into whorl of each seedling. Plants were inoculated in the evening to provide favourable conditions for establishment of infection.

Assessment : Qualitative evaluation

Assessments were conducted 21, 25 and 29 days after inoculation (d.a.i.). Lesions were classified on the basis of visual symptoms:

R^+ = chlorotic lesions without necrosis

R = chlorotic lesions with some necrosis

R⁻ = chlorotic lesion with considerable necrosis

S = wilted and necrotic lesion without chlorosis

(Hooker, 1977).

Assessment : Quantitative evaluation

For each plant a single lesion was identified and the increase in length monitored at 4 day intervals over a period of 12 days following the 21st d.a.i. Where possible only necrotic (susceptible type) lesions were measured. The percentage of total leaf tissue with blight symptoms (% LTB) was estimated for some leaves during each assessment.

Assessment : Sporulation

Three 1 cm^2 portions were cut from a NCLB lesion for each treatment at 28 d.a.i. The infected leaf pieces were placed on a moist filter paper in petri plates and incubated for 3 days at 25°C with 12 hour light/dark regime. On the fourth day, each leaf piece was put in a vial containing 2 ml of SDW and agitated vigorously for 1 minute. Sporulation was expressed as the number of conidia produced per leaf piece. A sporulation rating was devised by observing each leaf piece under a dissecting microscope. A scale of 0-5 was used:

0 = no conidia

1 = trace

2 = poor

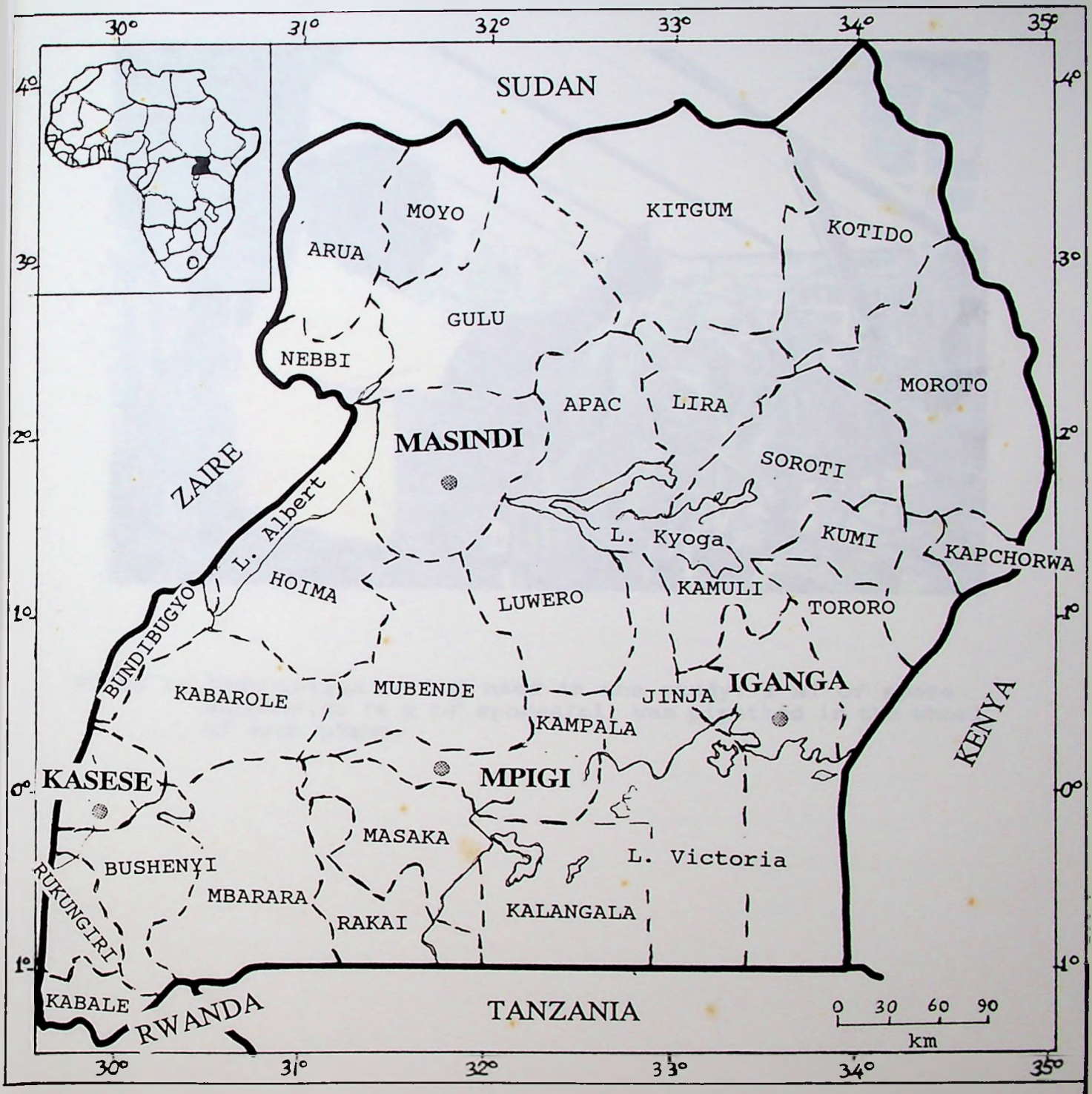
3 = intermediate

4 = good

5 = very good (Leath *et al.*, 1990).

Analysis of variance of lesion length, % LTB, sporulation rating and sporulation were worked out (INSTAT, University of Reading statistics package). Lesion length, % LTB and sporulation rating were regressed against sporulation ability.

FIGURE 1: MAP OF UGANDA SHOWING DISTRICTS WHERE *E. TURCICUM* ISOLATES WERE SAMPLED FROM.



Key ● districts sampled

— — — District boundary

Plate 1: Inoculation method used in the study: 1 ml of spore suspension (4×10^4 spores/ml) was pipetted in the whorl of each plant.

2.1.2 Experiment 2

Characterisation of Races of *E. turcicum* present in Uganda under controlled environmental conditions.

Objective: To compare results obtained from glasshouse trial with those obtained under controlled conditions.

Materials and Methods

Pathogen isolate: Due to limited space in the growth cabinet, only two isolates were used: Masindi (Ugandan) and 2N (US). Spore suspensions were prepared as for experiment 1.

Host plants: The five isogenic differential lines containing Ht resistance genes were used as for experiment 1. Two seeds were sown in peat based multipurpose compost in 9 cm diameter plastic pots. For each treatment (isolate x host) 3 replicate pots were sown. Seedlings were originally grown up in a glasshouse, but were transferred to a growth cabinet after inoculation. Twenty five day old plants were inoculated as for experiment 1. Inoculation was carried out in the evening in the glasshouse and inoculated plants were transferred the following morning to the growth cabinet where they remained up to the end of evaluations. Temperatures in the growth cabinet were set at 22°C day and 18°C night under 12 hour light/dark regimes (Leonard *et al.*, 1989; Thakur *et al.*, 1989; Leath *et al.*, 1990).

Assessment : Qualitative

Evaluations were conducted 14 d.a.i. Lesions were classified on the basis of visual symptoms:

N = necrotic, susceptible-type lesion

C = chlorotic, resistant-type lesion

2.1.3 Experiment 3

Susceptibility of Ugandan cultivars to isolates of *E. turcicum*

Objective: To evaluate/assess the susceptibility of Ugandan open pollinated varieties (OPV) and hybrids to *E. turcicum* isolates from Uganda and race 2 from the US.

Materials and Methods

Pathogen isolates: Two isolates Kasese (Ugandan) and 2N (USA) were used. Spore suspensions were prepared as for experiment 1, and concentrations adjusted to $4x10^4$ spores/ml before inoculation.

Host plants: Six Ugandan genotypes: Longe 1, EV. 8428SR, EV. 8429SR, Babungo (3), topcross 8556-6 and singlecross 8535-23 were evaluated. The first four are OPV whilst the last two are hybrids. Three seeds were sown in peat based multipurpose compost in 13 cm diameter plastic pots. For each treatment (isolate x cultivar) 4 replicate pots were sown. Following emergence, seedlings were thinned to 2 per

plot. Plants were grown in heated glasshouse with no additional lighting. 30 day old plants were inoculated as for experiment 1.

Assessment : Qualitative evaluation

Evaluations were conducted 21, 25 and 29 d.a.i. Lesions were classified on the basis of visual symptoms as in experiment 1.

Assessment : Quantitative evaluation

Assessments were carried out as for experiment 1, but in addition lesion number and incubation period were considered. Incubation period was determined by considering number of days from inoculation to when lesions measured more than 15 mm.

Assessment : Sporulation

Three 1 cm^2 portions from the NCLB lesions were excised 28 d.a.i. The leaf pieces were incubated on moist filter paper as for experiment 1 and number of conidia and sporulation rating assessed as for experiment 1 (after Leath *et al.*, 1990).

2.2 Growth rates *in vitro*

2.2.1 Experiment 4

Growth rates

Objective: To determine the effects of media composition and temperature on the radial growth rate of 6 isolates of *E. turcicum* in culture.

Materials and Methods

The radial growth rate in culture of 6 isolates: Iganga, Kasese, Masindi, Mpigi, USA (2N) and Zimbabwean were assessed on two media: LCH and Potato dextrose agar (PDA) (see appendix 2 for recipe).

Eight mm plugs were cut from the margins of 10 day old LCH cultures and placed mycelium-side-down in the centre of 9 mm plastic petri dishes containing LCH and PDA. Four replicated plates of each isolate were incubated in the dark at 2 temperatures: 23 and 28°C. Colony diameters were measured daily from day 3 after inoculation until growth of the colony in some treatments reached the edge of the plates. Radial growth rates were derived from the means of two diameters taken at right angles to each other for each colony each day. The diameters of the inoculum plug were subtracted from the total diameter recorded in each case. The 'rate' was then expressed for measurements taken in the exponential phase of growth only. The period of time from d.a.i to day 3 was considered as the lag phase; when the

isolate recovered from transfer.

2.2.2 Experiment 5

The effect of temperature on radial growth in culture.

Objective: To determine the growth rate of 3 isolates on 2 different types of media on 4 temperatures.

Materials and Methods

The radial growth rate in culture of 2 Ugandan isolates (Iganga and Kasese) and the Zimbabwean isolate were assessed on 2 media: LCH and PDA. Four temperatures: 15, 20, 25 and 30°C were tested.

Eight mm plugs of each isolate were placed mycelium side-down in the centre of 9 cm diameter plastic petri dishes containing the different media. Three replicate plates for each treatment were incubated in continuous light at 15, 20, 25 and 30°C. Colony diameters were measured daily starting from second day of inoculation (d.o.i) except for cultures at 15°C whose diameters were measured at two day interval. Growth rates were determined as for experiment 4.

2.2.3 Experiment 6

Radial growth rate in culture at 15°C of 3 isolates.

Objective: To compare growth rates of the remaining Ugandan isolates at 15°C with those derived from experiment 5.

Materials and Methods

The radial growth rate in culture of 2 Uganda isolates (Masindi and Mpigi) were assessed at 15°C on 2 media LCH and PDA. Inoculation, radial measurements and growth rates were determined as described in experiment 5.

2.3 Sporulation *in vitro*

2.3.1 Experiment 7

The effect of different light/dark regimes on the sporulation of *E. turcicum in vitro*.

Objective: To determine the optimum light/dark regime for sporulation.

Materials and Methods

Sporulation ability *in vitro* was assessed for 6 isolates: 4 Ugandan (Iganga, Kasese, Masindi and Mpigi); the Zimbabwean and the USA isolate (2N). The 3 light regimes used were: continuous light, 12 hour light/dark cycle and continuous dark. Cultures were set up as described in experiment 6 and incubated at 25°C under 3 light regimes.

3 ml of SDW were added to each 8 day old culture. Spores were dislodged from the medium surface using a dropper. Spore suspensions were filtered through a muslin cloth. Spore production for each treatment combination was determined using a haemocytometer.

CHAPTER 3

3.1 RESULTS AND DISCUSSION

3.1.1 Experiment 1

Three of the isogenic differential lines: H4460Ht1, H4460Ht2 and H4460Ht3 developed chlorotic lesions after inoculation with all isolates, rather than the classic blight lesion. Thus lesion length, % LTB, sporulation rating and sporulation were only determined on H4460 and H4460Ht1 which developed necrotic and a few chlorotic lesions with some necrosis respectively. The classic necrotic lesions were observed for line H4460 (no Ht resistance) against all isolates and in A697(W22HtN) inoculated with the US isolate. Reaction types are summarised in table 6.

Table 6: Reaction types of 5 isogenic corn lines containing the Ht genes for resistance to 6 isolates of *E. turcicum* from Uganda, Zimbabwe and the standard 2N from the US.

	ISOGENIC LINES				
ISOLATE	H4460	H4460Ht1	H4460Ht2	H4460Ht3	A697(W22HtN)
Ht gene	0	1	2	3	N
Masindi	S[a]	R	R^+	R^+	R^+
Kasese	R^+	R^+	R^+	R^+	R^+
Iganga	S	R^+	R^+	R^+	R^+
Mpigi	S	R^+	R^+	R^+	R^+
Zimbabwe	S	R^+	R^+	R^+	R^+
2N	S	R^+	R^+	R^+	S

a = as described in materials and methods in experiment 1.

Table 7: Summary of quantitative results 21, 25 and 29 d.a.i (all values are means of five replicates).

ISOLATE	DIFFERENTIAL LINE	21 d.a.i LESION LENGTH (mm)	21 d.a.i %LTB	25 d.a.i LESION LENGTH (mm)	25 d.a.i %LTB	29 d.a.i LESION LENGTH (mm)	29 d.a.i %LTB	SPORULATION RATING (0-5)	CONIDIA/ CM^2
MASINDI	H4460	82.6	18	110	26.4	185.2	47	4	8222
	H4460Ht1	59.2	8	86	15.6	108	23	0.3	178
	A697(W22HtN)	-[a]	-	-	-	-	-	-	-
KASESE	H4460	93	16	121	27.4	139.4	32.6	3.8	6344
	H4460Ht1	34.2	8	48.6	12.8	74.8	17.7	0	0
	A697(W22HtN)	-	-	-	-	-	-	-	-
IGANGA	H4460	76	17	102.6	22.6	147.4	29	4	6256
	H4460Ht1	50.6	8.6	75	15	95.8	20	0	0
	A697(W22HtN)	-	-	-	-	-	-	-	-
MPIGI	H4460	95.6	17	125.2	22	155.8	40.6	4	7289
	H4460Ht1	44	8.6	65.2	15	83.2	20	0.1	67
	A697(W22HtN)	-	-	-	-	-	-	-	-
ZIMBABWE	H4460	89	11.2	123	20.4	152.6	31.4	3.4	2200
	H4460Ht1	33.4	5.4	58.8	11.2	70	16.6	0	0
	A697(W22HtN)	-	-	-	-	-	-	-	-
US (2N)	H4460	58.8	7.4	80.2	11	114.6	19	2.3	1844
	H4460Ht1	27.2	5.6	48.2	8	54.2	12.4	0	0
	A697(W22HtN)	41.7	5.5	58.9	10	101	16	2	1093
SE		3.620	0.785	4.176	0.892	5.996	1.519	0.234	421.97

a not determined

Lesion length, % LTB, sporulation rating (figures 2, 3 & 4 respectively) were significantly different ($P<0.001$) for different isolates on the differential lines. Comparisons were made using analysis of variance (ANOVA) and values were highest for H4460 which did not contain the Ht gene. Interactions between differential lines and isolates were also significant ($P<0.001$) and it can be concluded that the two differential lines H4460 and H4460Ht1 react differently to the isolates tested.

Conidial production was significantly different on differential lines but less so between isolates (figure 4). This demonstrates that lesions formed on the susceptible line (H4460) produced more spores than the resistant line H4460Ht1 (see tables 8a-h). Necrotic lesions took slightly longer (14-21 days instead of 7-14 days) to develop on A697(W22HtN) compared to H4460 indicating that it was slightly less susceptible. Lesion lengths and sporulation scores demonstrated that the Masindi isolate was most aggressive. However, the two Ugandan isolates Masindi and Mpigi gave variable results on H4460Ht1 with a mixture of intermediate and susceptible type lesions forming.

The results obtained suggest that the isolates from Uganda and Zimbabwe are race 0. Thus a programme utilising monogenic resistance could successfully incorporate genes Ht1, Ht2, Ht3 and HtN. However, since selection for novel races with virulence to single Ht genes may cause breakdown

of resistance under field conditions inclusion of a form of polygenic resistance is important.

Lesion formation of 6 isolates of E. turcicum on two corn lines

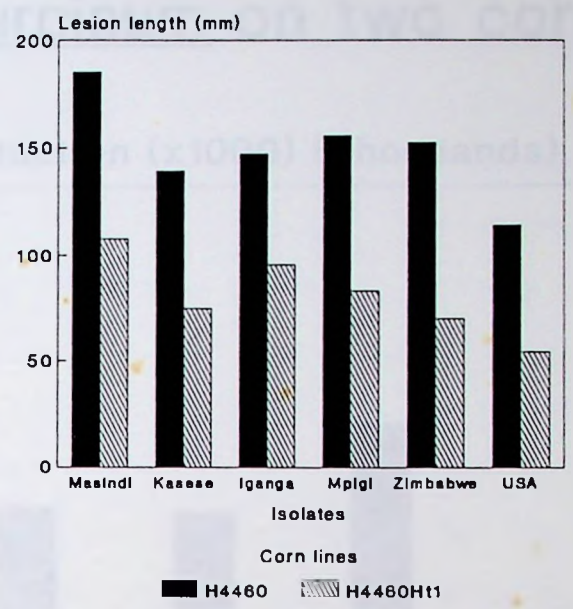


Figure 2

Pathogenicity of 6 isolates of E. turcicum on two corn lines

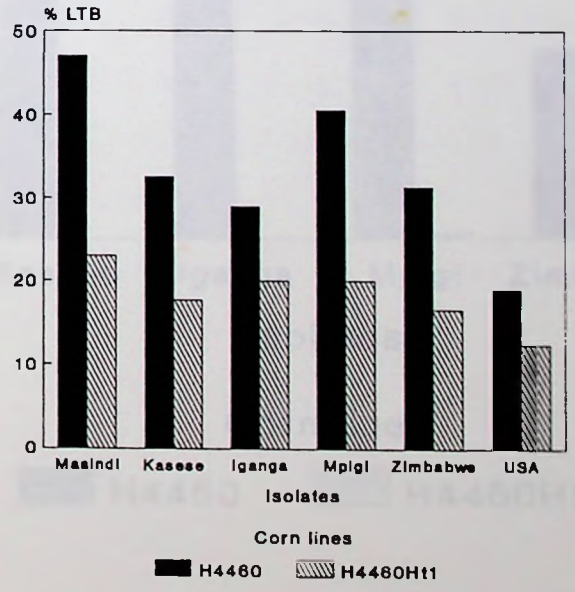


Figure 3

Sporulation of 6 isolates of E. turcicum on two corn lines

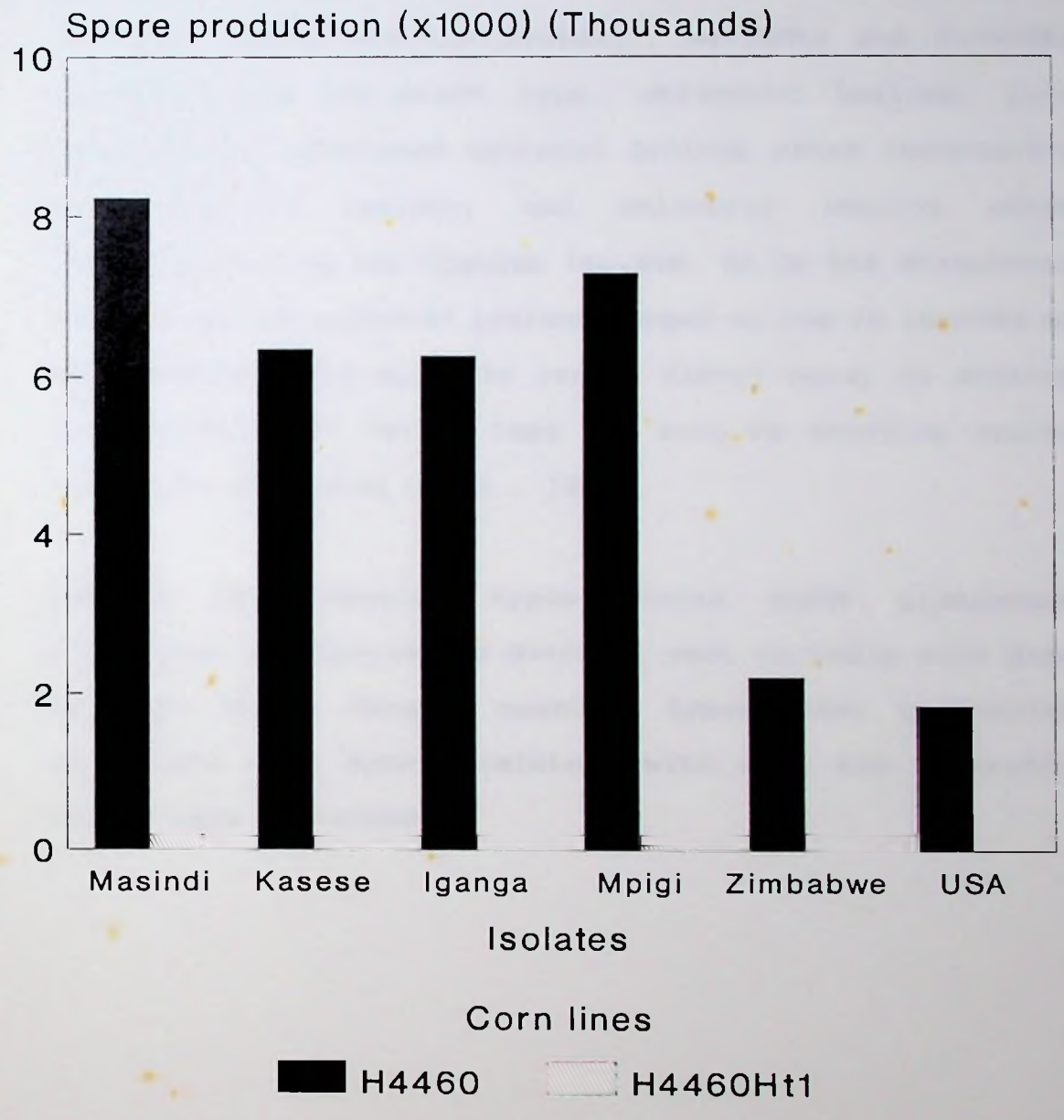


Figure 4

3.1.2 Experiment 2

The overall results obtained in the growth cabinet were found to be consistent with those obtained from the glasshouse (table 10). The universal susceptible line H4460 developed necrotic lesions after inoculation with both isolates tested whereas H4460Ht1, H4460Ht2 and H4460Ht3 developed the resistant type, chlorotic lesions. Line A697(W22HtN) developed necrotic lesions after inoculation with the US isolate, and chlorotic lesions after inoculation with the Ugandan isolate. As in the greenhouse experiment the necrotic lesions formed by the 2N isolate on A697(W22HtN) took slightly longer (10-14 days) to develop than on H4460 (7 days). Gene HtN acts by delaying lesion formation (Raymundo *et al.*, 1981).

Whereas the reaction types scored under glasshouse conditions for Masindi on H4460Ht1 were variable with some necrotic lesion formed, reaction types under controlled conditions were more consistent with only the chlorotic lesion type expressed.

Table 10: The reaction type of 5 maize differential lines containing the Ht resistance gene to two isolates of *E. turcicum*, the Ugandan Masindi and the US 2N 14 d.a.i under controlled conditions (18°/22°C; 12 hours dark/light).

	Differential line				
Isolate	H4460	H4460Ht1	H4460Ht2	H4460Ht3	A697(W22HtN)
Ht gene	0	1	2	3	N
Masindi	N	C	C	C	C
2N	N	C	C	C	N

N = necrotic susceptible type lesion

C = chlorotic resistant type lesion

3.1.3 Experiment 3

Susceptibility of Ugandan cultivars to isolates of *E. turcicum*.

Chlorotic lesions were observed on all cultivars inoculated with the US (2N) isolate except topcross 8556-6, Babungo (3), Longe 1 and singlecross 8535-23. Some plants (<10%) of varieties EV. 8428SR (population 28) and EV. 8429SR (population 29) developed a mixture of chlorotic lesions and lesions showing chlorosis and necrosis (table 11).

The classic susceptible necrotic lesions were observed on all six cultivars inoculated with the Ugandan isolate (Kasese). However, there were significant differences between cultivars with respect to lesion length, % LTB, number of lesions, sporulation rating and sporulation ($p<0.001$) (Tables 13a-j). Topcross 8556-6 was the most resistant with the least number of lesions, lesion length, % LTB, sporulation rating and sporulation. Longe 1, Babungo (3) and singlecross 8635-23 were ranked second most resistant while populations 28 and 29 were most susceptible with very high values for the five parameters assessed (figures 5-9). Incubation period was comparatively short in the susceptible cultivars, populations 28 and 29 with values of 7.3 and 7.8 days for the Kasese isolate; 17.8 and 19.8 days for the US 2N isolate respectively. In comparison with the topcross, which took 14 days with Kasese isolate and no lesion formation by isolate 2N.

There were positive correlations between assessments of lesion length, % LTB, lesion number, sporulation rating and sporulation as demonstrated by b and R^2 values (table 14).

Table 11: Reaction type of Ugandan cultivars 21, 25 and 29 d.a.i with *E. turcicum* isolates.

Cultivar	Isolate	Reaction		
		21 d.a.i	25 d.a.i	29 d.a.i
Babungo (3)	Kasese	S[a]	S	S
	2N	CF[b]	R^+	R^+
Longe 1	Kasese	S	S	S
	2N	CF	R^+	R^+
Topcross	Kasese	S	S	S
	2N	CF	CF	CF
Singlecross	Kasese	S	S	S
	2N	CF	R^+	R^+
Population 28	Kasese	S	S	S
	2N	R^+	R^+	R^+
Population 29	Kasese	S	S	S
	2N	R^+	R^+	R

a = as described in experiment 1

b = chlorotic fleck

Table 12: Summary of quantitative data taken 21, 25 and 29 d.a.i (All values are means of four replicates).

CULTIVAR	ISOLATE	LESION LENGTH (mm)	%LTB	No. OF LESIONS	LESION LENGTH (mm)	%LTB	NO. OF LESIONS	LESION LENGTH (mm)	%LTB	NO. OF LESIONS	INCUB. PERIOD[a]	SPORULATION RATING (0-5)	CONIDIA/ CM^2
Babungo(3)	Kasese	41	4.5	2.3	65.8	9.5	2.5	82	15	2.5	13	1.5	860
	2N	-	-	-	7.0	1.8	0.8	10.5	2.5	0.8	26	0	0
Longe 1	Kasese	38.3	6.8	2.5	52.8	9.3	2.5	67	11.3	2.5	13.7	0.9	417
	2N	-	-	-	2.3	0.5	0.3	4	0.5	0.3	29	0	0
Topcross	Kasese	20.8	3.8	1.8	33.3	6.3	1.8	40.5	9.8	1.8	14	1	595
	2N	-	-	-	-	-	-	-	-	-	-	-	-
Singlecross	Kasese	50	7.8	3	77.8	12.3	3.3	100.8	17.3	3.5	10.8	1.8	1022
	2N	-	-	-	7.5	1.3	0.5	11.8	2	0.5	25.5	0	0
Pop. 28	Kasese	94.3	9.5	4.3	125	22	4.8	179	33.3	5.5	7.3	3.3	3005
	2N	29	5	2.5	41.8	9.5	3.4	57.8	12.8	3.4	17.8	0.1	157
Pop. 29	Kasese	75.8	19.5	5.5	105	29.5	6.3	170	38.3	6.8	7.8	3.1	3105
	2N	16.3	4.5	2	29	21.3	2	37.3	8.3	2	19.8	0.1	46.3
SE		5.08	1.042	0.304	6.431	1.456	0.320	8.982	1.967	0.336	1.381	0.183	167.83

a = days from inoculation to when lesions greater than 15 mm had formed.

Lesion length on 6 Ugandan cultivars inoculated with 2 isolates of E. turc.

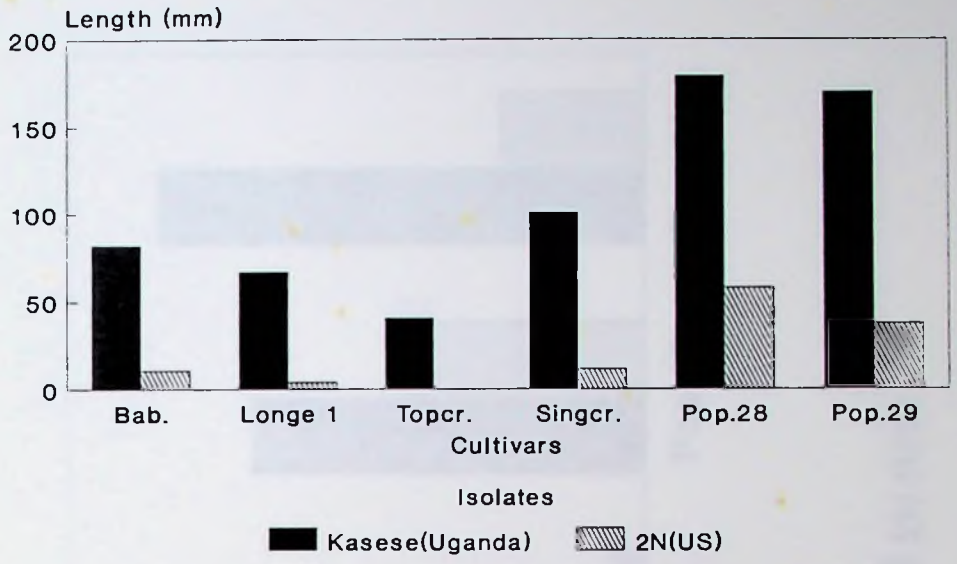


Figure 5

% LTB of 6 Ugandan cultivars inoculated with 2 isolates of E. turcicum

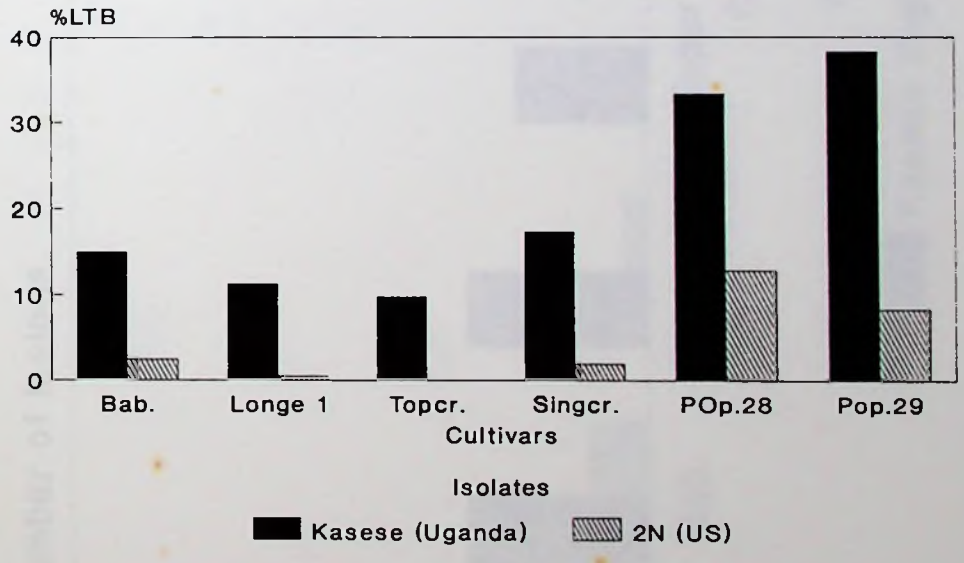


Figure 6

Number of lesions formed on 6 Ugandan cultivars inoc. with 2 isol. of E. turc.

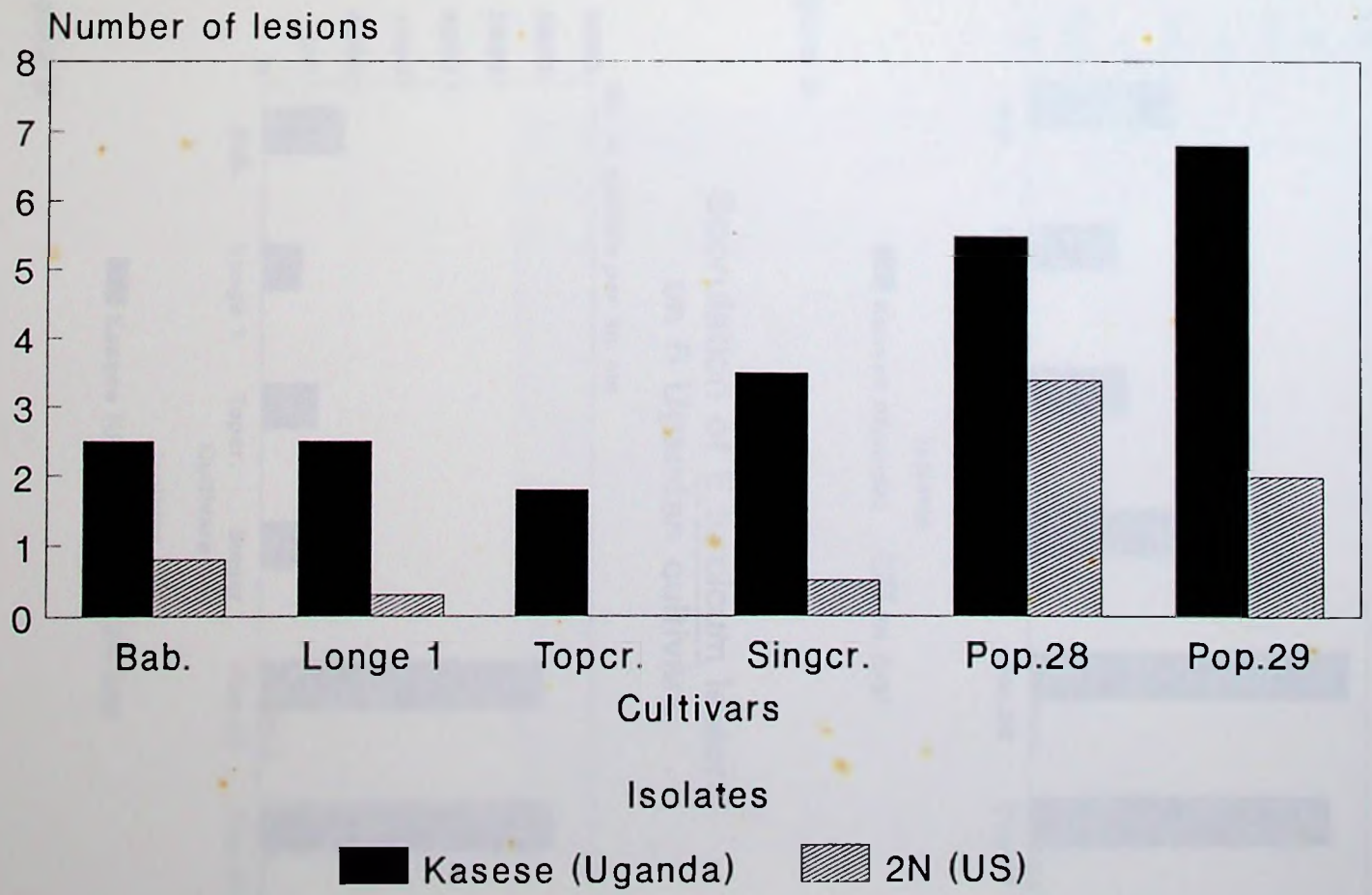


Figure 7

Sporulation rating of E. turcicum lesions on Ugandan cultivars

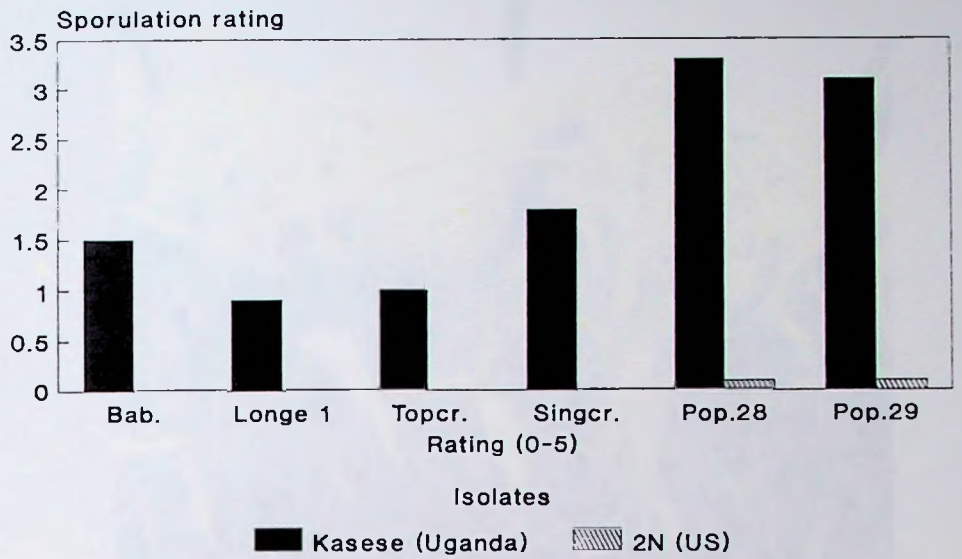


Figure 8

Sporulation of E.turcicum lesions on 6 Ugandan cultivars

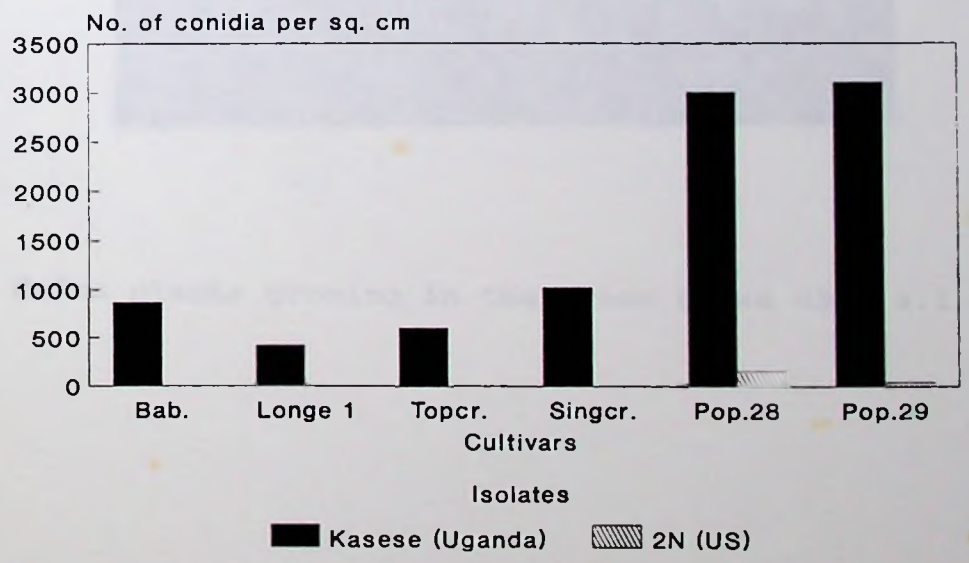


Figure 9

Plate 2: Maize plants growing in the green house 25 d.a.i.

Plate 3: Chlorotic resistant type lesions.

Plate 4: Classic necrotic susceptible type lesions.

Plate 5: Chlorotic flecks developed by some resistant genotypes.

3.1.4 Experiment 4

All isolates grew faster on PDA than on LCH at both temperatures tested. A higher radial growth rate was attained at 28°C than at 23°C. However, differences were observed between isolates. The Mpigi isolate had the highest radial growth rate on LCH at both temperatures but not on PDA, whereas the Iganga isolate had a more consistent pattern on both media at both temperatures. The Zimbabwean isolate appeared to grow better at 23°C than at 28°C on both media (figure 10-13). This indicates a possible difference in temperature optima.

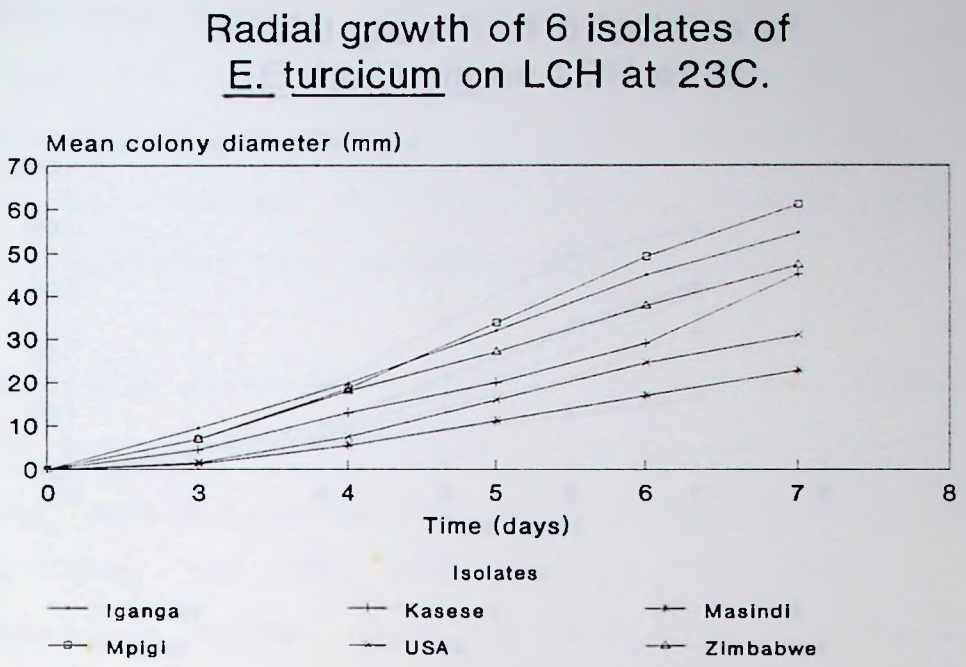


Figure 10

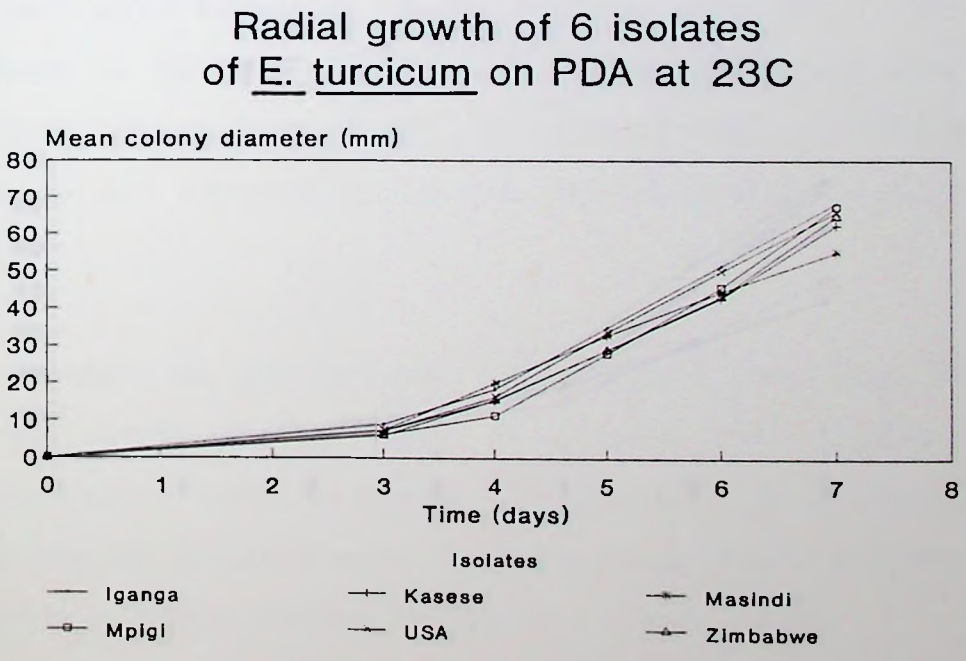


Figure 11

Radial growth of 6 isolates of E. turcicum on LCH at 28

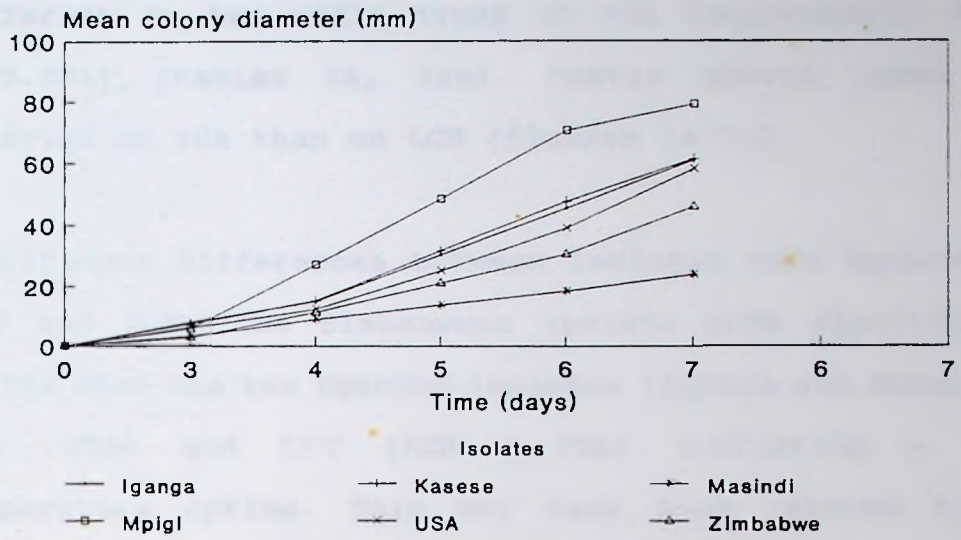


Figure 12

Radial growth of 6 isolates of E.turcicum on PDA at 28C

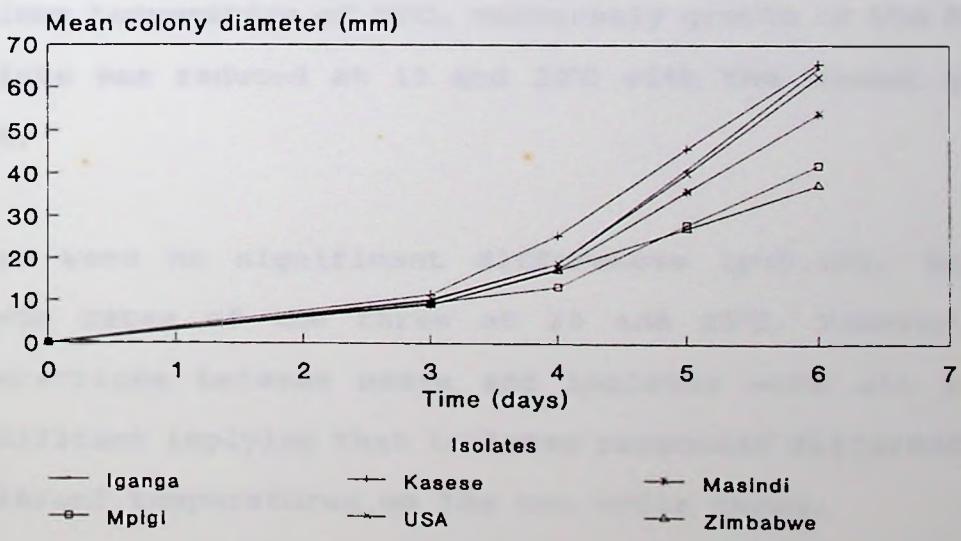


Figure 13

3.1.5 Experiment 5

Differences between the radial growth rates of the two Ugandan and the Zimbabwean isolates were significantly different on two media types at all temperatures tested ($p<0.001$) (tables 16, 16a). Faster growth rates were observed on PDA than on LCH (figures 14-21).

Significant differences between isolates were detected at 15°C and 30°C. The Zimbabwean isolate grew significantly faster than the two Ugandan isolates (Iganga and Kasese) at 20°C (PDA) and 15°C (LCH & PDA) indicating a lower temperature optima. This may have been related to the origin of the isolate, Henderson with temperature range of 8-26°C; which is generally cooler than in Uganda. The Kasese isolate grew faster at 25 and 30°C than the other isolates. Kasese is one of the hottest districts in Uganda with minimum temperature of 25°C, conversely growth of the Kasese isolate was reduced at 15 and 20°C with the lowest growth rate.

There were no significant differences ($p<0.001$) between growth rates of the three at 20 and 25°C. However, the interactions between media and isolates were all highly significant implying that isolates responded differently to different temperatures on the two media types.

Table 15: Mean growth rates mm/day of 3 isolates at 4 temperatures on 2 media types.

Isolate	Mean radial growth rate mm/day			
temp (°C)	15	20	25	30
Kasese	4.3[a](9.1)[b]	22.4(33.1)	23.4(33.6)	25(36.4)
Iganga	6.9 (10)	22.9(33.9)	24.7(32.6)	22.3(33.8)
Zimbabwe	9.5 (12.8)	21.6(34.8)	22.7(32.3)	19.6(28.4)

a = LCH; b = PDA

Radial growth of 3 selected isolates of E. turcicum on LCH at 15C

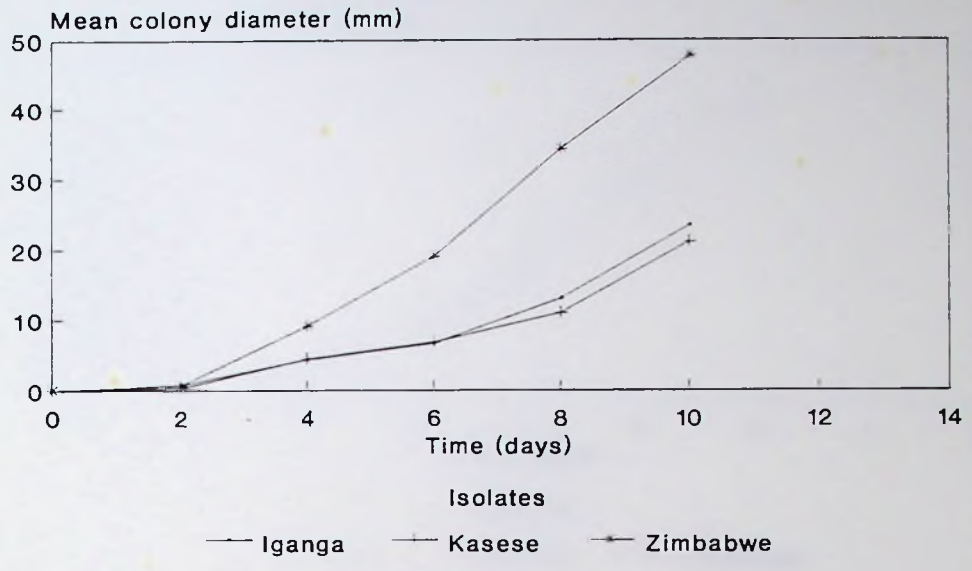


Figure 14

Radial growth of 3 selected isolates of E. turcicum on PDA at 15C

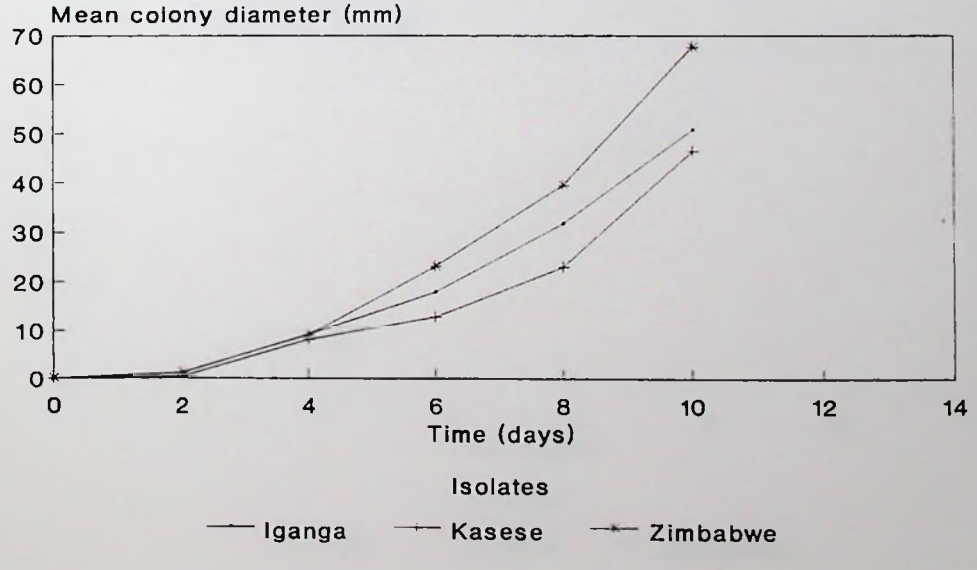


Figure 15

Radial growth of 3 selected isolates of E. turcicum on LCH at 20C

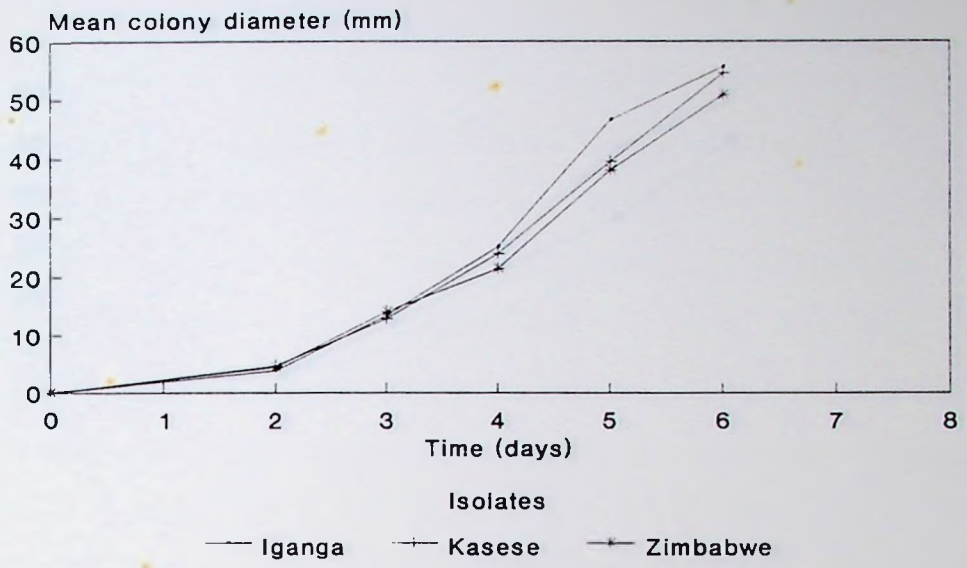


Figure 16

Radial growth of 3 seleicted isolates of E. turcicum on PDA at 20C.

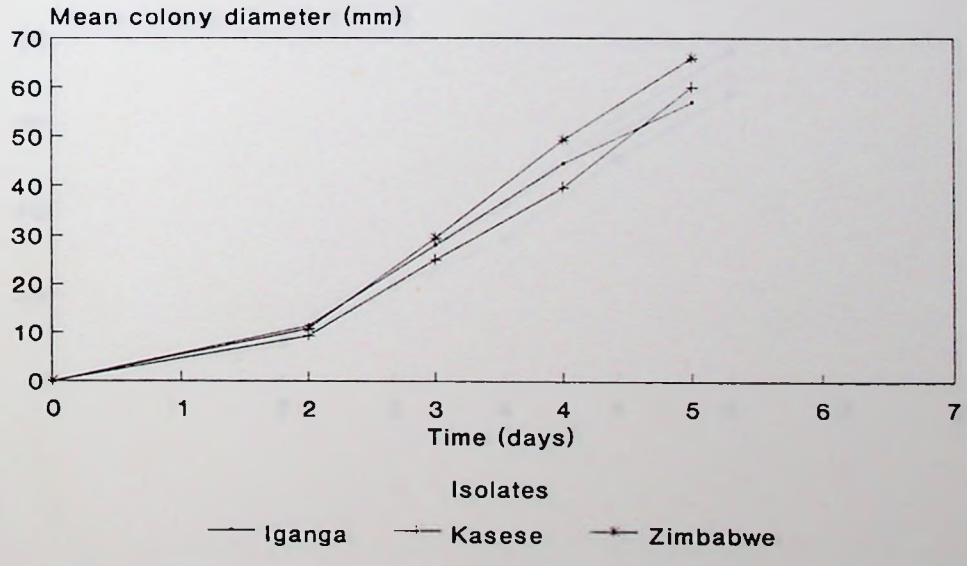


Figure 17

Radial growth of 3 selected isolates of E. turcicum on LCH at 25C.

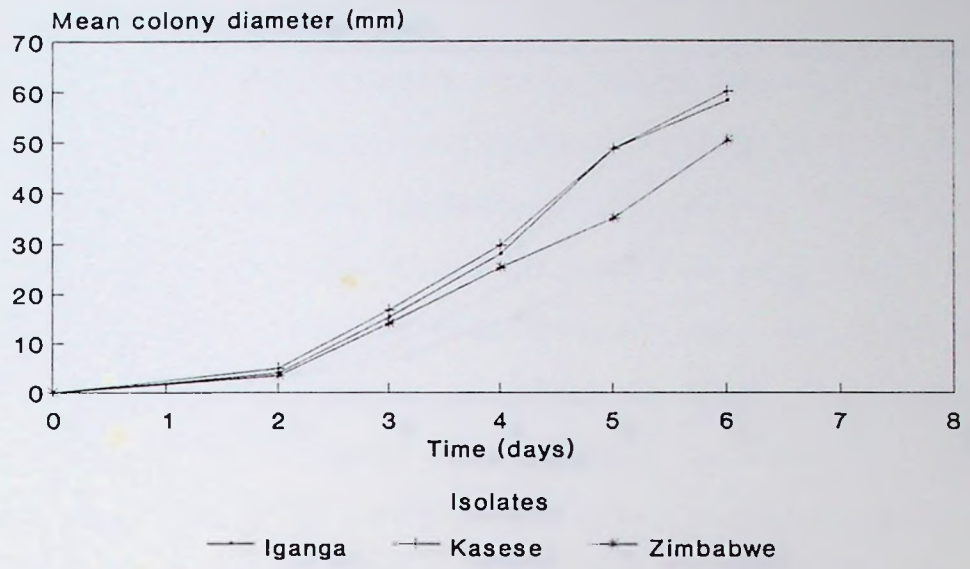


Figure 18

Radial growth of 3 selected isolates of E. turcicum on PDA at 25C.

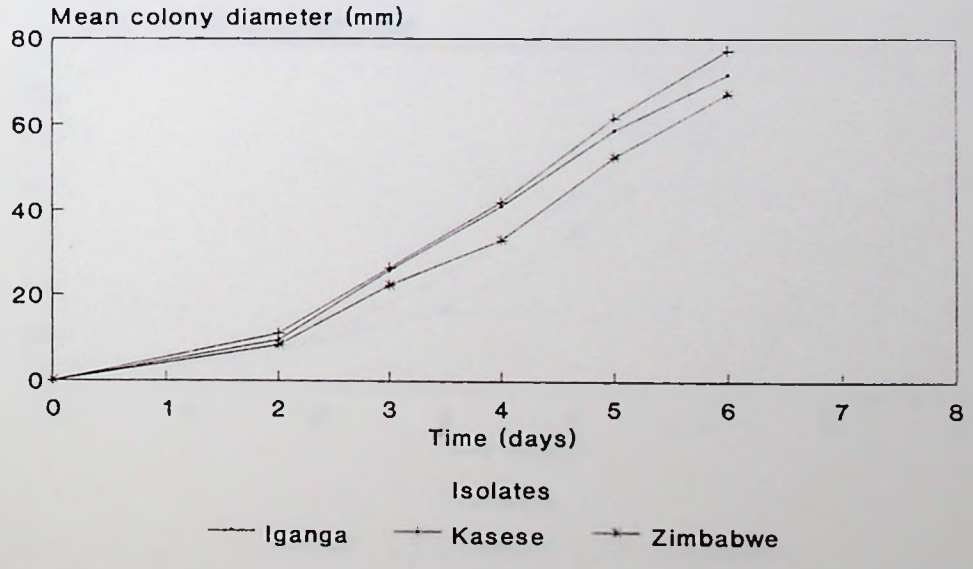


Figure 19

Radial growth of 3 selected isolates of E. turcicum on LCH at 30C.

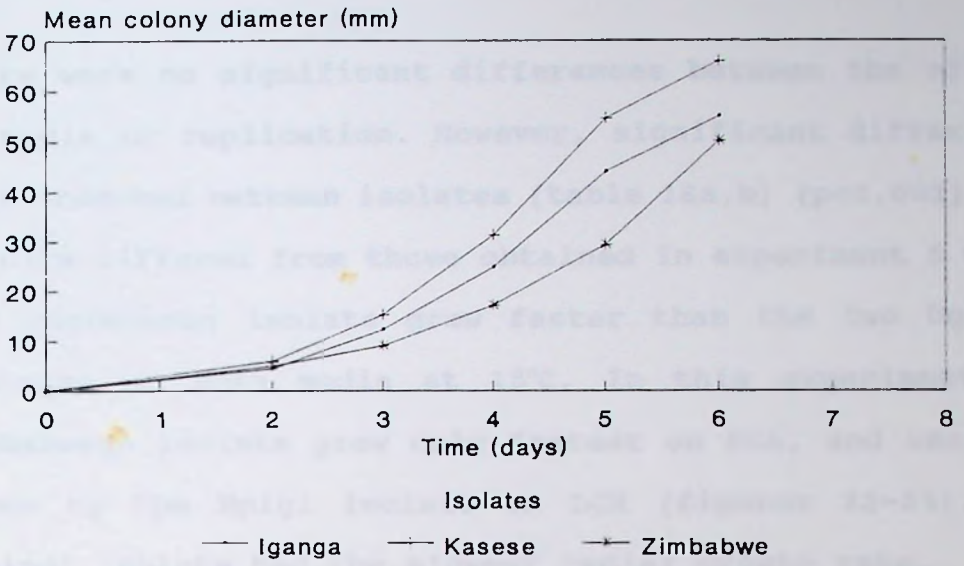


Figure 20

Radial growth of 3 selected isolates of E. turcicum on PDA at 30C.

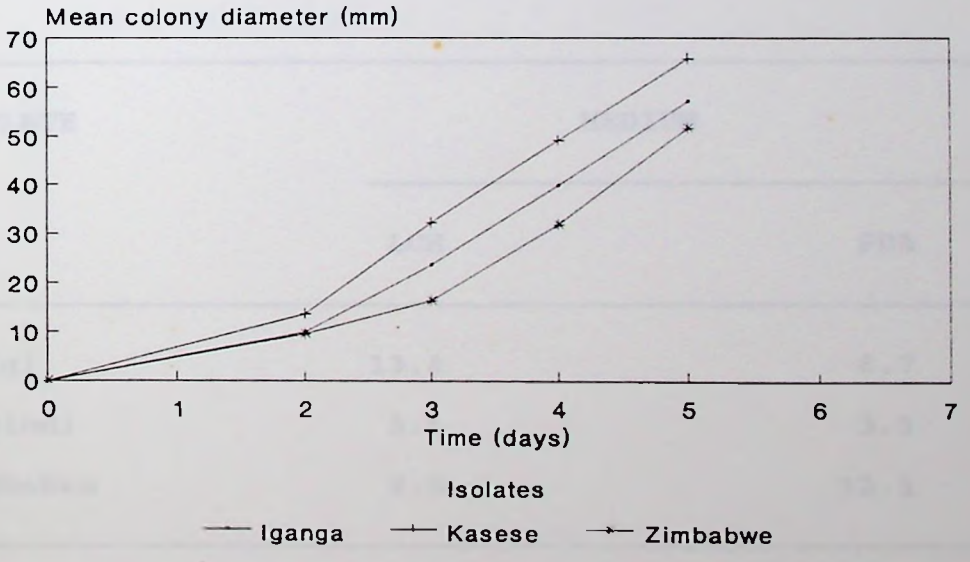


Figure 21

3.1.6 Experiment 6

Radial growth rate in culture of selected Ugandan isolates at 15°C.

There were no significant differences between the effects of media or replication. However, significant differences were observed between isolates (table 18a,b) ($p<0.001$). The results differed from those obtained in experiment 5 where the Zimbabwean isolate grew faster than the two Ugandan isolates on both media at 15°C. In this experiment the Zimbabwean isolate grew only fastest on PDA, and was out-grown by the Mpigi isolate on LCH (figures 22-23). The Masindi isolate had the slowest radial growth rate.

Table 17: Mean radial growth rate (mm/day)

ISOLATE	MEDIUM	
	LCH	PDA
Mpigi	13.4	8.7
Masindi	5.4	3.3
Zimbabwe	9.5	12.1

Radial growth of 3 selected isolates of E. turcicum on LCH at 15C.

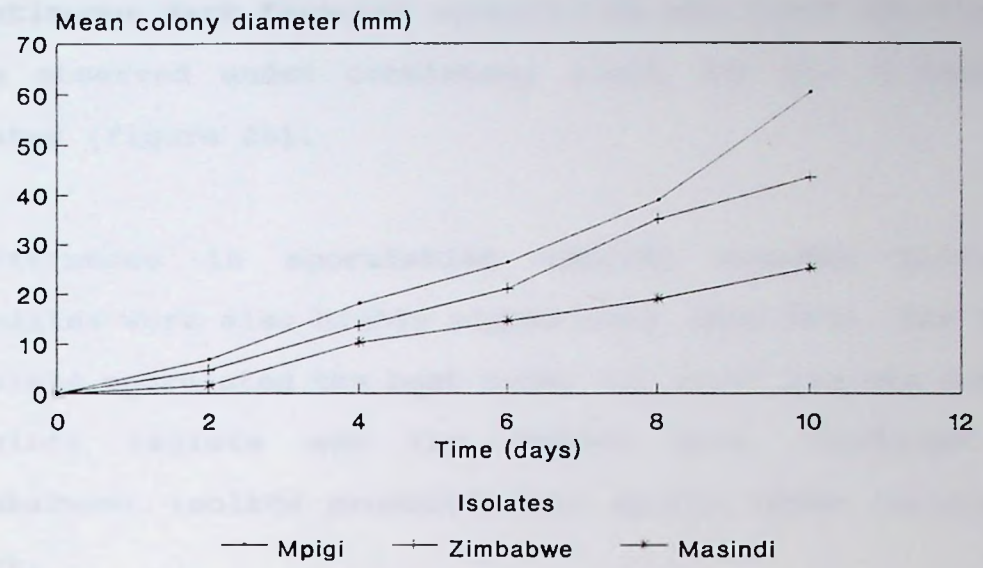


Figure 22

Radial growth of 3 selected isolates of E. turcicum on PDA at 15C.

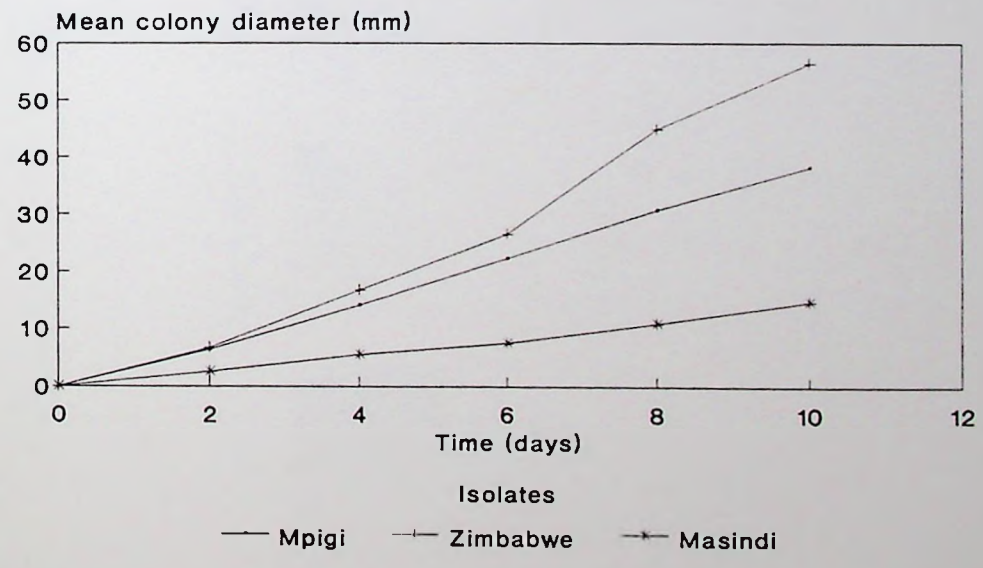


Figure 23

3.1.7 Experiment 7

The effects of different light regimes on sporulation were statistically significant ($p<0.001$) (table 19a,b). Continuous dark favoured sporulation and least sporulation was observed under continuous light for all 6 isolates tested (figure 24).

Differences in sporulation ability between different isolates were also highly significant ($p<0.001$). The US 2N isolate sporulated the best under all light regimes and the Masindi isolate was the second best, although the Zimbabwean isolate produced more spores under continuous dark.

The interaction effects of light and isolate were also highly significant ($p<0.001$).

Effect of different light/dark regime on sporulation of E.turcicum

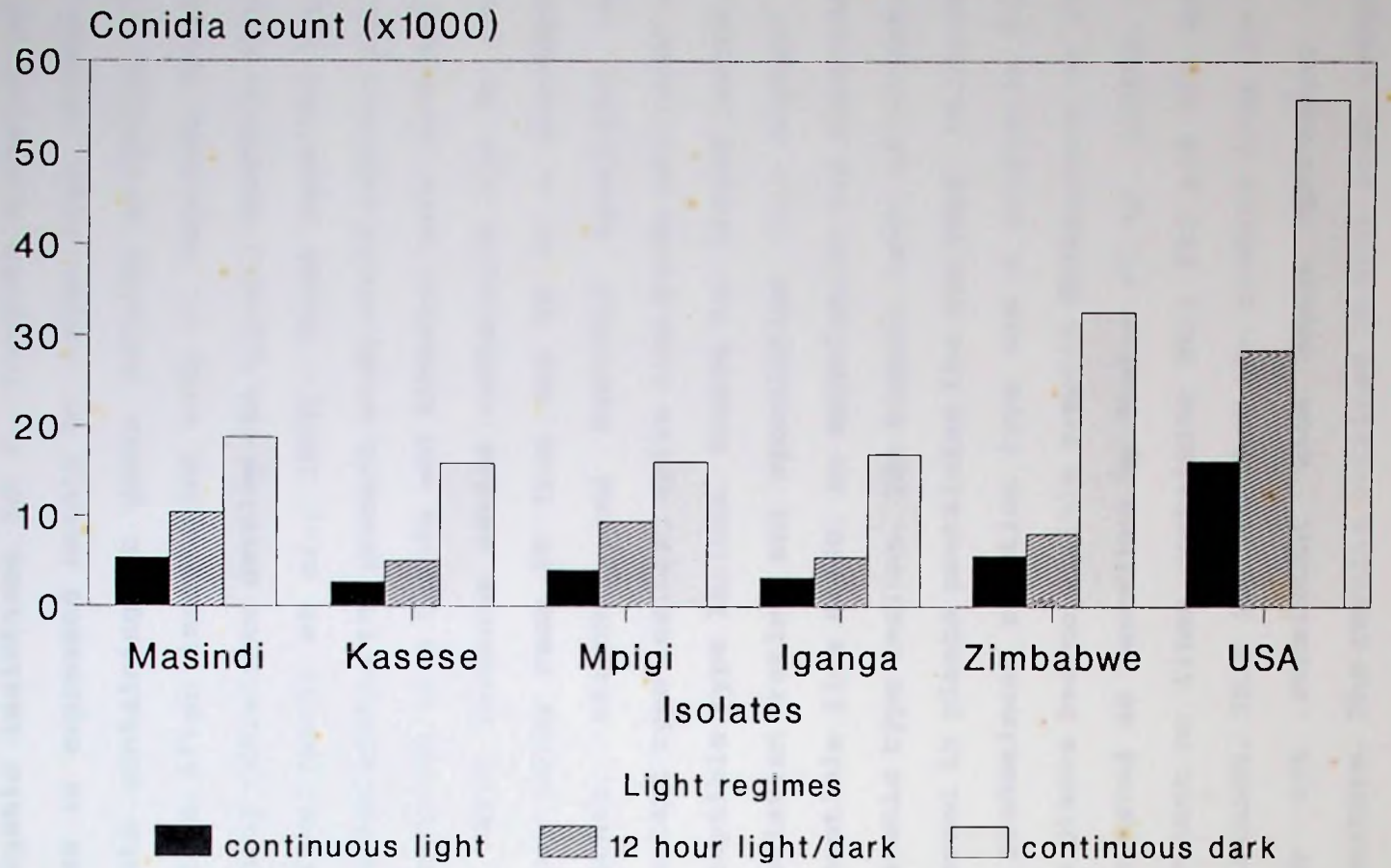


Figure 24

3.2 GENERAL DISCUSSION

Monogenic resistance to *E. turcicum* mediated by the Ht genes is expressed mainly by lesion type (Hooker, 1977). Plants containing Ht genes develop primarily chlorotic lesions after inoculation with *E. turcicum* whilst those without resistance develop the typical necrotic sporulating lesions (Abadi *et al.*, 1989). These reaction types were observed within the present study where African isolates of *E. turcicum* from Uganda and Zimbabwe were screened against the H4460 isogenic series containing the Ht resistance genes, using race 2N from the US as a standard. Lines H4460Ht1, H4460Ht2 and H4460Ht3 developed chlorotic resistant type lesions, while line H4460 developed necrotic susceptible type lesions. Scores for lesion length, % LTB, sporulation rating and sporulation were highest in the susceptible line H4460. No sporulation was observed in the chlorotic type lesions. The highest level of resistance was observed in plants containing the HtN gene. In these plants the predominant reaction type was a chlorotic fleck and some plants had no visible symptom development at all. Race 2N behaved as described by Thakur *et al.* (1989), and was avirulent on lines containing Ht1, Ht2 and Ht3 genes for resistance. This implies that the results from the current study are consistent with those described in the literature. The results obtained in this study suggest that race O may be the prevalent race in Uganda. To further confirm the prevalence of race O in Uganda, it is necessary

to screen a large number of isolates from different parts of the country. Tests with the Zimbabwean isolate indicated that this was also race 0, although a larger sample is also required to confirm this. Ideally experiments should be conducted under controlled environmental conditions as the variability in results derived from the greenhouse studies suggest that resistance may be breaking down at higher temperatures. This phenomenon was also observed by Leonard *et al.* (1989), and Thakur *et al.* (1989).

The expression of resistance by the HtN gene ie prolongation of incubation period with a subsequent delay in lesion formation offers a unique departure from other known types of resistance to *E. turcicum* (Raymundo *et al.*, 1981; Thakur *et al.*, 1989). In this study this type of reaction was demonstrated by line A697(W22HtN). Visible lesion development was also delayed on the resistant Ugandan cultivars eg. Topcross, Longe 1 and Babungo (3) when inoculated with isolate 2N, compared with the Ugandan Kasese isolate.

Sigulas *et al.* (1988), observed that the average level of resistance expressed as mean lesion area, the rate of increase in lesion size and lesion shape are strongly influenced by the host genotype. Results from this study support this statement. Lesions formed on H4460Ht1 were significantly narrower and longer than those formed on H4460. In addition the resistant Ugandan cultivars

(Topcross, Longe 1 and Babungo (3)) developed smaller lesions than the susceptible cultivars population 28 and 29.

Results from this study are consistent with the observations of Hilu and Hooker (1963) and Raymundo *et al.* (1981), increases in the number of lesions and the susceptible cultivars; population 28 and 29 had large numbers of lesions. In addition there was a positive correlation between lesion number, % LTB, incubation period, sporulation rating and sporulation. Susceptible cultivars had higher values of these parameters whilst values were lower for symptom expression on the resistant cultivars. The susceptibility of population 28 and 29 in the current study also supports the results from field experiments conducted in Uganda (Uganda national maize research annual reports 1988-91).

This study has identified some useful sources of resistance (topcross, Longe 1 and Babungo (3)) to Ugandan isolates of NCLB. Lesion length, % LTB, lesion number and incubation period appear to be reliable indices for assessing relative susceptibility / resistance.

As races have developed in US with virulence to genes Ht1, Ht2 and Ht3, the most effective use of monogenic sources of resistance in Uganda would be to combine genes Ht1 with Ht2 or Ht3 or HtN (Smith and Kinsey, 1980). The most probable

outcome of continued use of monogenic resistance, will be selection for new more virulent races of *E. turcicum* with a consequent demand for new resistance sources for their control. The most significant attributes of monogenic resistance are the suppression of sporulation, which reduces the production of secondary inoculum (Hilu and Hooker, 1963; Hooker and Lim, 1973; Ullstrup, 1970), reduces lesion size and prolongation of the latent period. Critical time for NCLB is 2 weeks after silking; shift in epidemic 6-8 weeks after silking would reduce yield losses (Hooker, 1977; Ullstrup, 1977).

The utilisation and incorporation of polygenic resistance sources has been limited by the difficulty of transferring multigenic resistance to inbreds without affecting agronomic characters such as consistency, harvest time and grain quality and moisture (Hooker and Lim, 1973). However, evidence reviewed here suggests that future research should be directed towards combining monogenic and polygenic resistance to control the occurrence and spread of *E. turcicum*.

Information on disease spread and progress is important for the prediction of epidemics (Fry, 1982). Empirical models using factors such as the area under the disease progress curve (AUDPC) and apparent infection rates could be developed to predict infection rates. It is also important in future to carry out yield loss studies.

E. turcicum is highly variable in culture. Single conidial progeny from a single conidial culture differs in colour, hyphal morphology, growth rate and sporulation in culture (Robert, 1952; Leonard, 1988). This study has demonstrated that growth and sporulation of *E. turcicum in vitro* are temperature and light dependant. Growth rate also varied according to the origin of the isolate. For instance, the Zimbabwean isolate from Henderson grew faster than all other isolates at lower temperatures ie 15 and 20°C. This suggests that it has a lower temperature optima. Conversely, the Kasese isolate derived from a hotter environment grew faster at higher temperatures ie 25, 28 and 30°C. Other isolates were intermediate. This suggests that isolates become adapted to their environment such that biotypes exist with different cardinal temperatures.

It has been reported that virulence does not correlate with growth rate *in vitro* (Leonard, 1988). This study has also demonstrated this; as the Masindi isolate with the lowest growth rate in culture was found to be most virulent on maize. Levy (1989) from his study on race characterisation in Israel suggested that aggressiveness was based on the lesion length and area, such that the isolate causing largest lesions was most aggressive. The results imply that growth rate in culture is not an indicator of pathogenicity *in vivo*.

As with growth rates in culture, sporulation is also

temperature and light dependant (Nisikado, 1927; Tuite, 1969; Sivanesan, 1985), with maximum production at temperatures of 24-26°C. Levy (1980) observed highest sporulation of *E. turcicum* under dark conditions at 22°C, whilst Sivanesan (1985) reported that *E. turcicum* sporulated better under 12 hour light/dark cycle with near ultra-violet light. However, this study supports the findings of Levy (1980), such that *E. turcicum* sporulated best in continuous darkness; with least sporulation in continuous light. The US (2N) isolate sporulated best of all isolates at all light regimes. Of the four Ugandan isolates, the Masindi isolate sporulated more *in vitro* than its counterparts while the Kasese isolate had the poorest sporulation. The Masindi isolate also produced the largest number of conidia *in vivo* from lesions produced on H4460 in experiment 1 whilst the Iganga isolate produced the lowest number of conidia. There is no clear correlation between sporulation in culture and sporulation on lesions *in vivo*.

Growth rate in culture, and sporulation appeared to be inversely linked such that isolates with lower radial growth rates produced most spores. However, spore production in culture may be correlated with sporulation in the host, and pathogenicity; and this may provide a rapid technique for pathogenicity testing. Further experimentation is required to clarify this.

APPENDICES

APPENDIX 1: Medium preparation

(a) Lactose Casein hydrolysate (LCH)

Lactose	37.5 g
Casein hydrolysate	3.0 g
Potassium dihydrogen phosphate (KH_2PO_4)	1.0 g
Magnesium sulphate ($MgSO_4.7H_2O$)	0.5 g
Microelements	2.0 ml
Oxoid agar No. 1	15.0 g

Dissolve in 1 litre of distilled water, and adjust pH to 6.0.

Microelements

Iron nitrate ($Fe(NO_3)_3.9H_2O$)	723.5 mg
Zinc sulphate ($ZnSO_4.7H_2O$)	439.8 mg
Manganese sulphate ($MnSO_4.4H_2O$)	203.0 mg

Dissolve microelements one at a time in a litre of water. Sulphuric acid added until the solution clears.

(b) Oxoid Potato Dextrose Agar

Oxoid potato dextrose agar	39.0 gm
Distilled water	1.0 l

APPENDIX 2: Abbreviations

cm centimetre

mm millimetre

ml millilitre

µm micrometre

Kg kilogramme

ha hectare

°C or C degree Celsius

% percent

g gram

mg milligram

l litre

APPENDIX 3: Others

AUDPC	Area under the disease progress curve
LCH	Lactose Casein hydrolysate
PDA	Potato Dextrose Agar
CIMMYT	Centro International de Memoramiento de Maiz y Trigo (International Maize and Wheat Improvement Centre)
NCLB	Northern corn leaf blight
SDW	Sterile distilled water
SED	Standard error of difference
LTB	Leaf tissue blighted
OPV	Open pollinated varieties
d.a.i	days after inoculation
d.o.i	days of inoculation
Expt.	Experiment
E. turcicum	*Exserohilum turcicum*
H. turcicum	*Helminthosporium turcicum*

APPENDIX 4: ANOVA tables

Table 8a: ANOVA table for lesion length in Experiment 1

Source	DF	SS	MS	F	Prob.
Differential	1	68074	68074	103.6	0.000
Isolate	5	21267	4253.5	6.5	0.000
Replicate	4	5702.5	1425.6	2.2	0.086
Error	49	32203	657.21		
Total	59	127247			

CV 22.3%

SED (differential) 6.619

SED (isolate) 11.46

Table 8c: ANOVA table for sporulation rating in Experiment 1

Source	DF	SS	MS	F	Prob.
Replicates	4	0.80833	0.20208	0.8	0.507
Differentials	1	173.4	173.4	720.8	0.000
Isolate	5	6.1833	1.2367	5.1	0.001
Error	49	11.792	0.24065		
Total	59	192.18			

CV 27.5%

SED (differential) 0.1267

SED (isolate) 0.2194

Table 8d: ANOVA table for number of conidia produced during Experiment 1

Source	DF	SS	MS	F	Prob.
Replicates	4	15434941	3858735	0.7	0.593
Differentials	1	519857422	519857422	94.9	0.000
Isolate	5	63397655	12679531	2.3	0.058
Error	49	268364699	5476831		
Total	59	867054716			

SED (differential) 604.3

SED (isolate) 1047

Table 8e: Interaction ANOVA table for lesion length in Experiment 1

Source	DF	SS	MS	F	Prob.
Differen. x isolate	11	91084	8280.3	11.0	0.000
Error	48	36164	753.41		
Total	59	127247			

Table 8f: Interaction ANOVA table for %LTB in Experiment 1

Source	DF	SS	MS	F	Prob.
Differen. x isolate	11	6051.4	550.13	12.4	0.000
Error	48	2135.2	44.483		
Total	59	8186.6			

Table 8g: Interaction ANOVA table for sporulation rating in Experiment 1

Source	DF	SS	MS	F	Prob.
Differen. x isolate	11	184.38	16.762	103.2	0.000
Error	48	7.8	0.1625		
Total	59	192.18			

Table 8h: Interaction ANOVA table for number of conidia in Experiment 1

Source	DF	SS	MS	F	Prob.
Differen. x isolate	11	641986550	58362414	12.4	0.000
Error	48	225068167	4688920		
Total	59	867054716			

Table 9: Estimates of parameters of linear regression of number of conidia in Experiment 1

Parameter	R^2	b	SE	t
Lesion length	0.4599	55.98	7.965	7.03^{***}
% LTB	0.3728	198.7	33.84	5.87^{***}
Sporulation rating	0.6099	165.9	174.2	9.52^{***}

Table 13a: ANOVA table for lesion length in experiment 3

Source	DF	SS	MS	F	Prob.
Replicate	3	537.5	179.17	0.2	0.895
Cultivar	5	60037	12007	13.5	0.000
Isolate	1	87552	87552	98.3	0.000
Error	38	33860	891.05		
Total	47	181987			

SED (cultivar) 14.93

SED (isolate) 8.617

Table 13b: Interaction ANOVA table for lesion length in experiment 3

Source	DF	SS	MS	F	Prob.
Cultivar x Isolate	11	160521	14593	24.5	0.000
Error	36	21466	596.26		
Total	47	181987			

Table 13c: ANOVA table for % LTB in experiment 3

Source	DF	SS	MS	F	Prob.
Replicate	3	91.167	32.389	0.5	0.716
Cultivar	5	2752.7	550.53	7.7	0.000
Isolate	1	3168.8	3168.8	44.5	0.000
Error	38	2708.1	71.265		
Total	47	8726.7			

SED (cultivar) 4.221

SED (isolate) 2.542

Table 13d: Interaction ANOVA table for % LTB in experiment 3

Source	DF	SS	MS	F	Prob.
Cultivar x Isolate	11	6534.7	594.06	9.8	0.000
Error	36	2192	60.889		
Total	47				

Table 13e: ANOVA table for number of lesions in experiment 3

Source	DF	SS	MS	F	Prob.
Replicate	3	7.0625	2.3542	1.2	0.340
Cultivar	5	91.854	18.371	9.0	0.000
Isolate	1	77.521	77.521	38.0	0.000
Error	38	77542	2.0406		
Total	47	253.98			

SED (cultivar)	0.7142
SED (isolate)	0.4124

Table 13f: Interaction ANOVA table for number of lesions in experiment 3

Source	DF	SS	MS	F	Prob.
Cultivar x Isolate	11	183.73	16.703	8.6	0.000
Error	36	70.25	1.9514		
Total	47	253.98			

Table 13g: ANOVA table for sporulating rating in experiment 3

Source	DF	SS	MS	F	Prob.
Replicate	3	1.2344	0.41146	0.9	0.467
Cultivar	5	12.714	2.5427	5.4	0.001
Isolate	1	43.13	43.13	90.8	0.000
Error	38	18.042	0.47478		
Total	47	75.12			

SED (cultivar) 0.3445

SED (isolate) 0.1989

Table 13h: Interaction ANOVA table for sporulating rating in experiment 3

Source	DF	SS	MS	F	Prob.
Cultivar x Isolate	11	65.932	5.9938	23.5	0.000
Error	36	9.1875	0.25521		
Total	47				

Table 13i: ANOVA table for sporulating (conidia/cm^2) in experiment 3

Source	DF	SS	MS	F	Prob.
Replicate	3	2246748	748916		
Cultivar	5	15016091	3003218		
Isolate	1	24670536	24670536		
Error	38	21646094	569634		
Total	47	63574969			

SED (cultivar) 377.4

SED (isolate) 217.9

Table 13j: Interaction ANOVA table for sporulating in experiment 3

Source	DF	SS	MS	F	Prob.
Cultivar x Isolate	11	52236279	4748753	15.1	0.000
Error	36	11343189	315089		
Total	47	63579469			

Table 14: Estimates of parameters of linear regression of number of conidia in experiment 3

Parameter	R^2	b	SE	t
Lesion length	0.6561	15.14	1.616	9.37^{***}
% LTB	0.5451	63.02	8.488	7.42^{***}
No. of lesions	0.5628	375.4	48.78	7.70^{***}
Sporulation rating	0.9237	888.2	37.47	23.60^{***}

Table 16a: ANOVA table for the radial growth rates of 3 isolates (Iganga, Kasese & Zimbabwe) at 15°C in experiment 5

Source	DF	SS	MS	F	Prob.
Medium	1	62.72	62.72	22.6	0.000
Isolate	2	60.248	30.124	10.8	0.002
Replicate	2	0.65778	0.32889	0.1	0.889
Error	12	33.339			
Total	17	156.96			

CV 19.0%

SED (medium) 0.7857

SED (isolate) 0.9623

Table 16b: The interaction between media and isolate at 15°C (experiment 5)

Source	DF	SS	MS	F	Prob.
Medium x Isolate	5	125.76	25.52	9.7	0.001
Error	12	31.207	2.6006		
Total	17	156.96			

Table 16c: ANOVA table for the radial growth rates of 3 isolates (Iganga, Kasese & Zimbabwe) at 20°C in experiment 5

Source	DF	SS	MS	F	Prob.
Medium	1	609.01	609.01	58.8	0.000
Isolate	2	1.3011	0.65056	0.1	0.939
Replicate	2	4.0878	2.0439	0.2	0.823
Error	12	124.24			
Total	17	738.65			

CV 11.6%

SED (medium) 1.54

SED (isolate) 1.886

Table 16d: The interaction between media and isolate at 20°C (experiment 5)

Source	DF	SS	MS	F	Prob.
Medium x Isolate	5	615.9	123.18	12.00	0.001
Error	12	122.73	10.228		
Total	17	738.63			

Table 16e: ANOVA table for the radial growth rates of 3 isolates (Iganga, Kasese & Zimbabwe) at 25°C in experiment 5

Source	DF	SS	MS	F	Prob.
Medium	1	384.57	384.57	36.0	0.000
Isolate	2	4.7478	2.3739	0.2	0.804
Replicate	2	3.6341	1.8172	0.2	0.845
Error	12	128.111	10.676		
Total	17				

CV 11.6%

SED (medium) 1.54

SED (isolate) 1.886

Table 16f: The interaction between media and isolate at 25°C (experiment 5)

Source	DF	SS	MS	F	Prob.
Medium x Isolate	5	125.78	25.52	9.70	0.001
Error	12	31.207	2.6006		

Table 16g: ANOVA table for the radial growth rates of 3 isolates (Iganga, Kasese & Zimbabwe) at 30°C in experiment 5

Source	DF	SS	MS	F	Prob.
Medium	1	500.33	500.33	66.2	0.000
Isolate	2	136.72	68.362	9.0	0.004
Replicate	2	17.008	8.5039	1.1	0.357
Error	12	90.73	7.5608		
Total	17	744.8			

CV 10.0%

SED (medium) 1.296

SED (isolate) 1.588

Table 16h: The interaction between media and isolate at 30°C (experiment 5)

Source	DF	SS	MS	F	Prob.
Medium x Isolate	5	644.17	128.83	1.54	0.000
Error	12	100.63	8.3856		
Total	17	744.8			

Table 16i: ANOVA table for the radial growth rates of 3 isolates (combined analysis) for experiment 5

Source	DF	SS	MS	F	Prob.
Medium	1	1392.2	1392.2	115.3	0.000
Isolate	2	7.9825	3.9912	0.3	0.720
	3	4982.7	1660.9	137.6	0.000
	2	2.2633	1.1317	0.1	0.911
Error	63	760.6	12.073		
Total	71				

CV 15%

SED (medium) 0.819

SED (isolate) 1.003

SED (temperature) 1.158

Table 16j: The interaction between medium, isolate and temperature (combined analysis) for experiment 5

Source	DF	SS	MS	F	Prob.
Medium x isolate x temp	7	6541.2	934.45	98.9	0.000
Error	64	604.53	9.4458		
Total	71	7145.7			

Table 18a: ANOVA table for the radial growth rate of 3 selected isolates (Masindi, Mpigi & Zimbabwe) at 15°C (experiment 6)

Source	DF	SS	MS	F	Prob.
Medium	1	8.82	8.82	1.2	0.297
Isolate	2	170.44	85.222	11.5	0.002
Replicate	2	0.70333	0.35167	0.0	0.954
Error	12	88.873	7.4061		
Total	17	268.84			

SED (medium) 1.283

SED (isolate) 1.57

Table 18b: The interaction between media and isolate for 3 selected isolates (Masindi, Mpigi & Zimbabwe) at 15°C (experiment 6)

Source	DF	SS	MS	F	Prob.
Medium x isolate	5	220.33	44.067	10.9	0.000
Error	12	48.507	4.0422		
Total	17	268.84			

Table 19a: ANOVA table for different light regimes on sporulation of *E. turcicum* (experiment 7)

Source	DF	SS	MS	F	Prob.
Light	2	3848956994	1924478497	31.3	0.000
Isolate	5	4261131433	852226286	13.9	0.000
Replicate	2	202559723	101279861	1.6	0.204
Error	44	2703413966	61441227		
Total	53	1.10160621E10			

SED (light) 2613

SED (isolate) 3695

Table 19b: The interaction between light and isolate (experiment 7)

Source	DF	SS	MS	F	Prob.
Light x isolate	17	9199526634	541148626	10.7	0.000
Error	36	1816535480	50459319		
Total	53	1.10160621E10			

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