CROP PROTECTION PROGRAMME

Management strategies for maize grey leaf spot (*Cercospora zeae-maydis*) in Kenya and Zimbabwe

DFID No. R7566 (NR International No. ZA 0371)

FINAL TECHNICAL REPORT

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DFID project reference number R7566; Crop Protection Research Programme.

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Abbreviations

AFLP – Amplification Fragment Length Polymorphism
ART – Agricultural Research Trust
CABI – CAB *International*
CABI-ARC - CAB *International* – Africa Regional Centre
CABI-UKC - CAB *International* – UK Centre
CFU – Commercial Farmers Union
CIAT – Centro Internacionale Agricoletura Tropical
CIMMYT - International Maize and Wheat Improvement Centre
CPP – Crop Protection Programme
DAF – Direct Amplification Fingerprinting
DFID – Department for International Development
DGGE - Denaturing Gradient Gel Electrophoresis
DNA – Deoxyribose Nucleic Acid
GLS – Grey leaf spot
ISSR – Internal Simple Sequence Repeat Polymorphism
ITS-RFLP – Internal Transcribed Spacer Restriction Fragment Length Polymorphism
KARI – Kenya Agricultural Research Institute
PCR – Polymerase Chain Reaction
PPRI – Plant Protection Research Institute
RAPDS – Random Amplified Polymorphic DNA
RFLP - Restriction Fragment Length Polymorphism
USA – United States of America
ZCFU – Zimbabwe Commercial Farmers Union

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Executive Summary

Grey leaf spot (GLS), caused by *Cercospora zeae-maydis* and, to a lesser extent, *Cercospora sorghi* var. *maydis,* is recognised as a major constraint to global maize production. The disease was first described on maize in the USA in 1924 and now occurs wherever the crop is grown. In Africa, the first report of GLS came from South Africa during the mid-1980's, from where it has spread northwards into the major maize-growing regions of the continent (Plate 1). Yield losses ranging between 30% and 60% have been reported, and the disease is considered to pose a serious threat to food security in Africa.

GLS was first reported in Kenya and Zimbabwe during the 1995 growing season, when small-scale maize farmers experienced significant yield losses. At the time, most of the maize varieties being grown were highly susceptible to the disease. Small-scale maize farmers have continued to experience considerable yield losses, particularly in GLS 'hot spots' and in some areas have been relying heavily on the use of fungicides to control the disease. GLS-resistant varieties, which are competitively priced and early maturing are now available, however, there appears to be a marked reluctance amongst small-scale farmers to switch to GLS-tolerant varieties.

The current project was developed in response to a call from DFID's Crop Protection Programme in 1999. The 'purpose' of the project was to provide effective management of GLS based on sound epidemiological principles. This was achieved through the successful delivery of five main project outputs: 1. Variability of the pathogen population determined using representative isolates from both Kenya and Zimbabwe; 2. Cultural practices affecting GLS incidence and severity identified; 3. Disease epidemiology established through field experimentation and supported by the use of molecular markers; 4. Effective host resistance screening based on pathogen variability; and 5. Improved cultural practices for disease control validated on farm. The outputs were accomplished through a range of research activities including surveys (biological and socio-economic), pathogenicity studies, use of molecular techniques, host screening and field-based epidemiological studies undertaken in close collaboration with the partner institutes i.e. Kenya Agricultural Research Institute (KARI), Kenya and the Plant Protection Research Institute (PPRI), Department of Research and Scientific Services, Zimbabwe.

Plate 1: Severe infestation by GLS

Knowledge generated by the project concerning the epidemiology of GLS, together with existing literature, has been used to design, develop and promote an integrated pest management strategy for GLS. The strategy is based on the use of cultural methods for the removal of crop debris, soil fertility and recommendations concerning host resistance. Promotional activities undertaken by the project include stakeholder workshops, interactive sessions between farmers and project researchers, and a series of participatory training exercises (with >50 extension staff and >500 small-scale maize farmers). Promotional materials include the production of a poster on 'raising awareness of maize GLS' which was disseminated to >5000 small-scale farmers and extensionists, a leaflet on 'options for managing maize grey leaf spot,' disseminated to >1500 small-scale farmers and extensionists, training video's on 'recognising GLS' and 'farmers perceptions of the disease,' and a published report of, 'A socio-economic survey of maize GLS on small-scale maize farms in Kenya and Zimbabwe.'

Background

Grey leaf spot (GLS) caused by the fungus, *Cercospora zeae-maydis* Tehon & E.Y Daniels, and to a much lesser extent by *Cercospora sorghi* var. *maydis*, was first described on maize in Illinois, USA in 1924 (Tehon and Daniels, 1925). It is currently recognized as one of most important, yield-limiting diseases of maize worldwide (Latterell and Rossi, 1983; Lipps *et al.*, 1998; Ward & Nowell, 1998), and is prevalent throughout the USA, Central America, Europe, South-East Asia, India, China and Africa (Ward *et al.*, 1999).

The first reports of GLS in Africa, came from South Africa in 1988 (Ward, 1996). Initially, the main foci for GLS was in KwaZulu Natal Province in South Africa, from where it has spread northwards into the major maize-growing regions of the continent (Ward *et al*., 1999). It was reported in Kenya and Zimbabwe during the 1995 growing season (Kung'u and Boa, 1997; Nowell, 1997), together with reports from Cameroon, Democratic Republic of Congo, Malawi, Mozambique, Nigeria, Swaziland, Tanzania, Uganda and Zambia (Nowell, 1997). Since its emergence in Africa, GLS has become an increasingly important constraint to maize production, and currently poses a serious threat to food security in Africa.

Yield losses ranging between 30% and 60% have been reported in Kenya, Zimbabwe and South Africa (Ward *et al*., 1996; ZCFU, 1997; Muriithi and Gathama, 1998; Ward *et al*., 1999). However, research in other maize producing countries suggests that if infection occurs early and favourable environmental conditions exist following infection, the disease is capable of causing total yield loss, particularly in cases of severe blighting, stalk deterioration and lodging (Latterell and Rossi, 1983; Saghai-Maroof *et al*., 1993; Gevers and Lake, 1994). Even where yield data have not been recorded, high severity levels of GLS are considered to indicate high yield losses due to reduced photosynthetic leaf area (Ward *et al*., 1999), which is particularly important at the grain-fill stage (Allison and Watson, 1996).

At the time GLS was first observed in Kenya and Zimbabwe, the maize varieties grown by small-scale farmers were particularly susceptible to the disease (Plate 2). Prior to 1995, GLS had not been reported in either country, which implies that *C. zeae-maydis* (or a newer strain) has been introduced into the region or, it has "evolved" in relation to existing maize hybrids, other hosts and/or a changing environment. Unconfirmed reports suggest that GLS may have been introduced into Africa from the USA through infested residue in aid shipments of maize imported during the drought years of the 1980's (Ward *et al*., 1999). However, following an analysis of isolates from various countries in Africa, alongside isolates from the USA, Dunkle and Levy (2000) rejected this hypothesis. Using data from an earlier study (Wang *et al*., 1998), which proposed the existence of two genetically distinct populations of *C*. *zeae-maydis* i.e. Groups I and II, it was established that isolates from Africa belonged exclusively to Group II. In the USA, the Group II population of *C. zeae-maydis* is reported to be confined to the eastern third of the country, which is not an area where white maize for human consumption is produced, making exports from this area to Africa highly unlikely (Dunkle and Levy, 2000).

Plate 2: Symptoms of GLS on maize.

Cercospora zeae-maydis is a polycyclic, facultative pathogen (Chupp, 1953; Stromberg and Donahue, 1986). Most reports suggest that it is only capable of infecting maize (Stromberg and Donahue, 1986), although Shurtleff (1980) suggests that Sorghum may also be a host. The pathogen overwinters in infested crop debris (Beckman and Payne, 1982; Latterell and Rossi, 1983), and this is widely believed to be the primary source of inoculum (Beckman and Payne, 1982; Stromberg and Donahue, 1986). The subsequent development of epidemics of GLS depends on

environmental conditions i.e. disease development requires a high relative humidity (>95%), cool, cloudy conditions with an extended dew period (Latterell and Rossi, 1983; Anderson, 1995) and temperatures of 22-30^{o}C (Beckman and Payne, 1982). Infections appear initially as pin-point, yellow lesions which elongate to form characteristic, rectangular lesions, delineated by the leaf veins, with a symptomatic 'grey' hue when held up to the light (Beckman and Payne, 1982). Symptoms usually develop on the lower leaves of the maize plant initially, and as more lesions form, individual lesions coalesce, resulting in severe leaf blighting of the whole plant (Stromberg and Donahue, 1986; Ward and Nowell, 1997). Early initiation of leaf blighting results in severe lodging and even further grain loss as a result of the reduction in harvestable yield.

Management strategies for GLS include the use of fungicides, cultural practices and resistant varieties. Reports of fungicide efficacy from the USA are variable (see for e.g. Ayers *et al.,* 1985; Smith, 1989; Wegulo *et al.,* 1997) although fungicides belonging to the benzimidazole and triazole chemical groups have been shown to provide good control (Caldwell, 2000). When GLS first emerged in Africa, fungicides were used widely by farmers in Southern Africa, in their initial attempts at managing the disease. However, the cost of fungicides, is beyond the reach of most small-scale maize farmers in Africa.

Attention has, therefore, turned towards the role of cultural practices in managing GLS. A number of reports have shown that *C. zeae-maydis* can only survive up to two years in crop debris (see for e.g. Latterell and Rossi, 1983; Ward *et al.,* 1993), so cultural practices which reduce crop debris are likely to reduce and/or delay the onset of GLS. In the USA, both crop rotation and deep ploughing have been shown to be effective in managing GLS (Latterell and Rossi, 1983; Stromberg and Donahue, 1986). However, crop rotation is not an option for many small-scale maize farmers in Africa, particularly if the value of the 'alternate' crop is lower than maize (Caldwell, 2000). Such practices are, in any case, only effective in areas where GLS is not endemic. Date of planting has been shown to affect the development of GLS (Rupe *et al.,* 1982), but rainfall patterns in most of Africa preclude the possibility of avoiding peak rainfall periods without compromising crop yield. One attractive possibility is the use of early maturing varieties, which tend to be preferred by small-scale farmers, are less susceptible to GLS (Ward *et al.,* 1997a) and may, therefore, be a valuable component in an integrated management strategy. Finally, there are contradictory reports concerning the notion that GLS is a 'rich farmers disease,' associated with areas of high soil fertility (N) (Smith, 1989; Caldwell, 2000; Carrera and Grybauskas, 1993). This aspect of the epidemiology of GLS warrants further investigation.

Of perhaps most interest, however, is the availability of host resistance which is potentially the most cost-effective method for managing maize GLS. Following the initial outbreaks of GLS in Africa, breeding programmes were undertaken by the maize seed companies, and GLS-tolerant varieties first appeared on the market in Southern Africa in 1997. Initially, these varieties were expensive, lower-yielding, late-maturing varieties, and therefore inappropriate for small-scale maize farmers. In the past two years, this situation has changed, with the increasing availability of affordable, higher yielding, and early maturing varieties. Nevertheless, there continues to be a marked reluctance amongst some small-scale maize farmers to switch to GLS-tolerant varieties.

The current project was developed in response to a call from DFID's Crop Protection Programme in 1999. By investigating variability in the pathogenicity of *C. zeae-maydis*, sources of inoculum, cultural practices affecting the incidence and spread of the disease, and the potential for using host resistance, the project aims to develop and promote an effective and appropriate, integrated management strategy for GLS by small-scale maize farmers, based on sound epidemiological principles.

Project Purpose

To provide effective management of grey leaf spot (GLS) of maize based on sound epidemiological principles, and to facilitate the selection of durable host resistance against the disease.

Small-scale maize farmers in Kenya and Zimbabwe are currently experiencing significant yield losses as a result of GLS (<40%). In order to develop improved methods for controlling the disease, and thereby contribute to the research goal, the epidemiology of the pathogens(s) must be ascertained, so that appropriate management strategies can be developed and promoted.

Research Activities

1.1 Survey to obtain fungal isolates from Kenya and Zimbabwe

Extensive biological surveys of the maize-growing regions in different agro-ecological zones of Kenya and Zimbabwe (Appendices 1 and 2) were carried out in September-October 2000 (Kenya), March-April 2001 (Zimbabwe) and June-July 2002 (Kenya). The majority of maize crops were surveyed at the tassling stage, when expression of GLS is known to be optimal. Prevailing conditions prevented a 'follow-up' survey in Zimbabwe, which was scheduled to take place in February 2003.

A 'rapid sampling' protocol was developed whereby small-scale maize farms were sampled at random, approximately every 20km along accessible roads and paths through the key maize-growing areas – the exact location and altitude was recorded using a geographic positioning system (GPS). A total of 550 small-scale farms were sampled (250 in Kenya and 300 in Zimbabwe). On each farm, at least 200 maize plants were sampled randomly along a transect(s) through the crop, and scored for the incidence and severity of GLS. The severity of GLS was assessed using the following categories; Trace <2% of leaf area infected, Low = 2-10% of leaf area infected, Moderate = 11-50% of leaf area infected, and Severe >50% of leaf area infected. The average score per farm was assessed on the basis of the sample plants. Diseased plant material (>500 specimens) was then collected from symptomatic maize plants and stubble at each farm. The diseased plant material was stored in individual paper bags until it could be examined in the laboratory. Data were entered into a Microsoft Excel spreadsheet, and the distribution and severity of GLS in each country was mapped using the package, MapInfo Professional™.

1.2 Isolation, identification and culturing of fungal isolates

In the laboratory, individual lesions on the diseased plant material were examined for sporulation, and using a sterile needle, synnemata were isolated initially onto tap water agar (TWA), and subsequently sub-cultured onto Vegetable Juice (V8) agar. A total of 372 isolates were single-spored and established in culture. The morphological and cultural characteristics of the resulting cultures were examined to determine species identity i.e. using conidiophore and spore morphology.

Most of the isolates obtained from the samples conformed to descriptions for *C. zeae-maydis*, although some lesions yielded only *C. sorghi*. Taxonomic identifications were carried out by CAB *International*. This extensive population of isolates from maize GLS was expanded to include isolates of *C. zeae-maydis* from other maize-producing countries (USA, South Africa, China) and related species of *Cercospora* including *C. beticola, C. longines* and *C. sorghi,* held in the CAB *International* Genetic Resources Collection and through contributions from other collaborative institutes. In total, >250 representative isolates were obtained from GLS lesions, and are currently being maintained in culture.

1.3 Assess pathogen variability in a representative sub-set of isolates, using direct amplification fingerprinting (DAF) and where appropriate sequence-characterized amplification reactions

Potentially one of the most challenging aspects of the molecular studies was developing culturing protocols for DNA extraction and DNA extraction methods, before establishing whether the extracted DNA was amenable to PCR analysis. Using representative isolates, a successful protocol for the production of mycelia and DNA extraction was developed by Dr Julian Smith, CABI-ARC, and subsequently used by Mr Z.M. Kinyua (KARI) and Mrs E. Mtisi (PPRI) during a three-month training attachment at CABI-UKC (April-June, 2001), to extract DNA from >75 isolates each from Kenya and Zimbabwe.

Morphological characterisation

Prior to analysing the genetic diversity of the isolates, a pilot study was undertaken to ascertain the most appropriate methodologies for studying species, sub-species and intra-species genetic diversity amongst the isolates. The approaches taken to analyse the extracted DNA included Restriction Fragment Length Polymorphism of the internal transcribed spacer region between ITS regions 1 and 4 (ITS-RFLP), RAPDs, Internal Simple Sequence Repeat Polymorphism (ISSR) and Arbitrary Fragment Length Polymorphism (AFLP). Consideration was given to the appropriateness of the method for subsequent transfer to the molecular biology facilities in each of the respective partner countries. In this context, a comparison was undertaken between AFLP methods based on agarose (2 base pair selective primers to Pst1 sites) and polyacrylamide (2 base pair selective to Eco R1/ Msp 1 sites): the former being readily transferable, whereas the latter requires DNA sequencing equipment not currently available at the partner institutes in Kenya or Zimbabwe. Full details of the methodologies and results of these studies can be found in project reports (Kinyua, 2001; Kinyua, 2002; Kinyua, 2003; Mtisi, 2001) for Kenya and Zimbabwe respectively.

Genetic diversity of isolates of maize GLS

Representative isolates of maize GLS from the populations of Kenya and Zimbabwe (>75 from each country) were characterised for their genetic variability by ITS-RFLP (electrophoresis after Taq*I* digestion of ITS-PCR products, produced through ITS region amplification of genomic DNA with primers ITS1 and ITS4) and AFLP with 2 bp selective primers to the Eco R1/Msp 1 sites, resolved on a polyacrylamide gel. Data were analysed using Gel Compare *II* software (Applied Maths BVBA, Belgium), which is designed to explore the relatedness of banding patterns.

Species and sub-species descriptions

Analysis of the ITS-RFLP concurred with morphological criteria on species descriptions for *C. zeae-maydis* and *C. sorghi*. Isolates with a 550bp ITS-PCR product were identified as either *C. zeae-maydis* or *C. sorghi.* The 550bp ITS-PCR product from each isolate was digested into two main fragments, in addition to other smaller fragments. Of the isolates characterised, those grouped as *C. zeae-maydis* yielded fragments approximately 170bp and 100bp in length while those identified as *C. sorghi* produced 220bp and 100bp fragments.

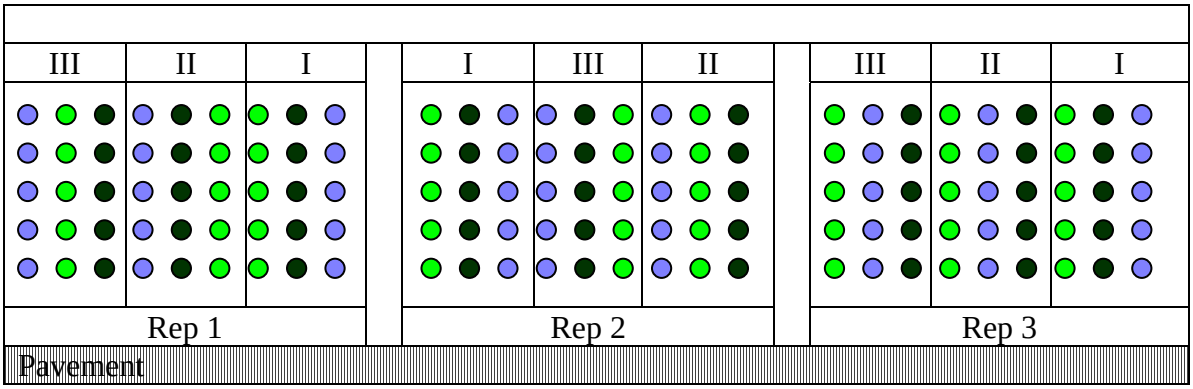
Intra-species genetic diversity

Intra-specific genetic diversity studies on the isolates of *C. zeae-maydis* were carried out by electrophoresis of AFLP-PCR products on either agarose or polyacrylamide (sequencing) gels (Kinyua, 2001).

1.4 Compare results against pathotyping studies to determine extent of evidence for pathotypic lineages.

Pathogenicity studies were carried out using detached leaf tests in the laboratory and artificial inoculation of intact plants in a screenhouse (KARI-NARL) to identify potential differences in the pathogenicity of isolates. A sub-set of isolates from Kenya (64 isolates) and Zimbabwe (48 isolates) were compared on healthy, leaf-pieces of a GLS-susceptible variety. Inoculum was prepared from pure cultures of each isolate which were grown for 14 days on V8 juice agar. Conidia from the cultures were harvested separately by flooding the culture plates with distilled water and dislodging the spores with a scalpel. The spore suspensions were then strained through cheesecloth and adjusted to a concentration of $1.8x10^4$ conidia/ml in a 0.01% Tween-20 solution, using a haemocytometer. One drop (0.1 ml) of each spore suspension was placed on a leaf piece, laid on moist filter paper in a Petri dish, sealed and incubated for four weeks. Three replicates were prepared for each isolate. Dimensions of GLS lesions were recorded every two weeks.

A screenhouse experiment was designed to investigate the virulence of isolates of *C. zeae-maydis* from two contrasting maize-growing regions in Kenya, with different agro-climatic conditions and different levels of GLS incidence. Isolates from Kakamega (No. 17) – a disease 'hot-spot' and Mtwapa in the Coastal Province (No. 31) which has a low incidence of GLS, were used in the a preliminary assessment (Figure 1). Molecular characterisation of both isolates by AFLPs revealed some minor genetic differences, with a DNA band difference at the 580bp position, albeit extremely small. To avoid the possibility of cross-contamination by other sources of GLS inoculum, the experiment was carried out in a non-GLS area. Three maize hybrids (Pioneer 3253, H511 and H614D) were planted in a split-plot design with 3 replicates; isolates and uninoculated controls formed the main plots and maize hybrids constituted the sub-plots, which were subsequently inoculated with an isolate. Inoculation took place 40 days after planting using hand-sprayed, inoculum suspensions of the respective isolates (~10ml spore suspension per plant). Data for the incidence and severity of GLS were recorded throughout the growing season. Yield data were also taken for green maize by measuring the cob lengths and weights.



Maize germplasm codes **1 = Phb 3253** **2 = H511** **3 = H614D**
GLS isolates: I (17), II (31), III ('None').
Planted: 25/3/2002; Inoculated: 3/5/2002; Uncovered: 6/5/2002

Figure 1. Experimental layout for GLS virulence and screening protocol (March 2002)

2.1 Socio-economic survey of maize-based, small-scale farms in Kenya and Zimbabwe to identify potential beneficial cultural practices.

A socio-economic survey of 219 (119 in Kenya and 100 in Zimbabwe) small-scale, maize farms was undertaken in selected maize-growing areas, where GLS was prevalent (CABI-ARC, 2002). Data from the biological survey of maize growing areas in Kenya and Zimbabwe (Activity 1.1) were used to identify key GLS areas – Western Kenya and Natural Regions IIA, IIB and III in Zimbabwe (Figure 2).

Plate 3: Socio-economic survey of GLS in Zimbabwe

The socio-economic survey had three specific objectives (Plate 3):

- Determine farmers' perceptions of the effect of different cultural practices on the incidence and severity of grey leaf spot on maize.
- Obtain baseline data on farmers' perceptions of yield loss attributable to grey leaf spot.
- Identify other insect pests and diseases affecting maize production.

Cultural practices on maize farms were recorded in relation to disease prevalence, to identify which indigenous cultural practices may have an effect on the incidence of maize grey leaf spot. Farmers perception of yield loss from GLS was also recorded together with the incidence of other insect pests and diseases affecting maize production, particularly where there exists a possible interaction with maize grey leaf spot.

Data collection questionnaires were developed for use in the farmer interviews (Appendices 3 and 4). Surveys for the two countries were similar, but with minor adjustments to take into account the differing cropping systems and land distribution characteristics. The surveys were carried out in February 2001 in Kenya and April 2001 in Zimbabwe. Data collection in Zimbabwe benefited from the fact that on most farms visited the maize crop was at a mature growth stage, enabling scientists to confirm the presence of GLS. In Kenya the maize had, in most cases, been harvested prior to data collection.

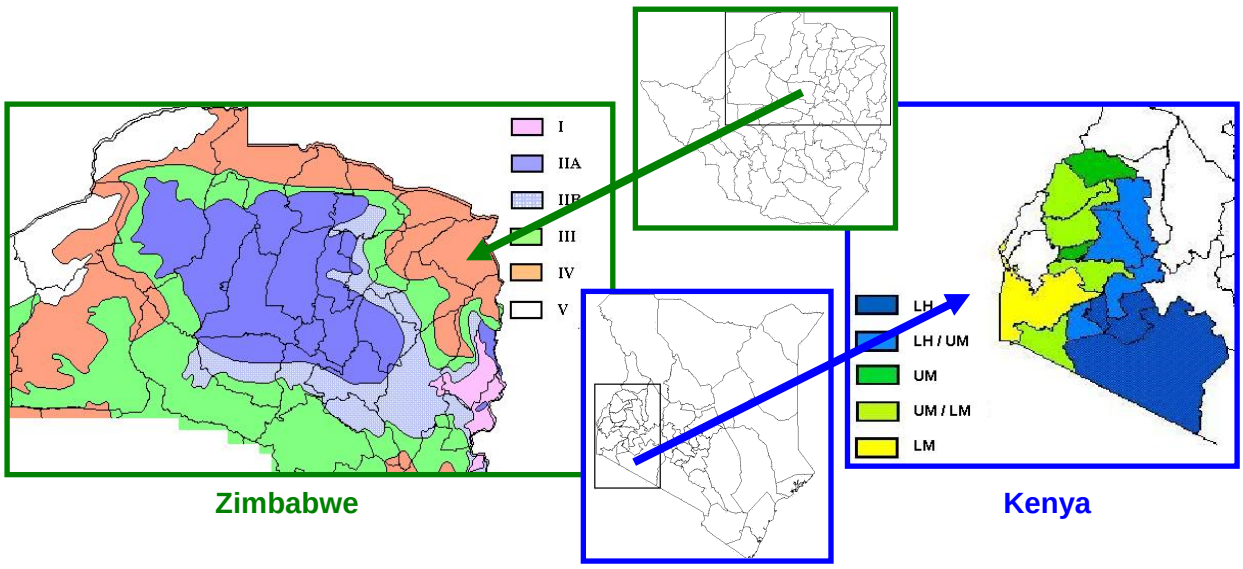


Figure 2: Areas of Zimbabwe and Kenya visited during the socio-economic surveys

Data were entered into a Microsoft Excel Spreadsheet, and summarised using Pivot tables and graphs. Information gathered during the surveys has been compiled together with a review of existing literature on GLS and data on maize production economics, and published (CABI-ARC, 2002).

2.2 Determine the effect of different cultural practices on the incidence and severity of disease, using survey data with collation of existing data from KARI, PPRI & South Africa

Literature searches using a number of international agricultural databases including CAB *International* Pest Abstracts, CAB *International* Crop Protection Compendium, Agricola, AGRIS (FAO) and TROPAG and RURAL, revealed that there are currently, there are >200 publications on GLS in Africa and other maize-growing countries around the world, primarily the USA. Publications deemed to be relevant to the current project were obtained wherever possible and reviewed. Dr

Julian Ward (formerly of KwaZulu Natal Department of Agriculture, South Africa) who has been at the forefront of GLS research in Africa kindly contributed all publications and grey literature from South Africa. Data on previous survey work undertaken by KARI under DFID NARP 1 and 2 and from studies undertaken by CFU and PPRI in Zimbabwe were collated and summarised to establish key intervention points and potential constraints to farmer adoption of disease management practices.

Data from existing literature on GLS were combined with data from the socio-economic surveys (Activity 2.1) and analysed using GENSTAT (Genstat 5, 1998) to determine the effect of cultural practices on the incidence and severity of GLS. These included investigations into relationships between the effects of maize variety, management practice (pre- and post-harvest) and other insect pests and diseases on the incidence and severity of GLS. Details on the effect of cultural practices on the incidence and severity of GLS can be found in CABI-ARC (2002).

3.1 Field epidemiological studies to ascertain key sources of inoculum

Alternative hosts

Potential alternative hosts of C. zeae-maydis were collected from Kenya and Zimbabwe during the biological surveys and subsequent field trials. Plants adjacent to GLS-infested maize crops were examined for possible symptoms of GLS in situ, and where symptoms resembling maize GLS were observed, representative samples were taken. In total, more than 50 samples were taken from 15 potential alternative hosts of C. zeae-maydis including sorghum, 'wild' sorghum, star grass, Guatemala grass, pearl millet, wild oat, Napier grass, Sudan grass, Bana grass, sugarcane and Columbus grass.

Isolates were cultured (as per Activity 1.2), and those that did not conform to morphological descriptions of Cercospora spp. were discarded. Molecular characterisation of the 14 remaining isolates using ITS-RFLP analyses confirmed their identity as C. sorghi. In addition, all 14 isolates were subsequently analysed by AFLP and shown to be of a similar order of diversity as recorded from the isolates of C. sorghi from maize. These isolates together with some representative isolates of C. zeae maydis and C. sorghi from maize are currently being DNA-sequenced between the ITS 1 and 4 regions to substantiate the analyses. C. zeae-maydis was not isolated from any potential alternative hosts. Moreover, artificial inoculation of C. zeae-maydis onto an array of 14 potential alternate hosts failed to induce GLS-like symptoms.

Imported grain

The Coast Regional Station of the Kenya Plant Health Inspectorate Service in Mombasa was visited in order to sample consignments of maize grain. Numerous samples were taken from imported yellow corn from the USA, together with pre-export maize produced in Kenya. Microscopic examination failed to detect the presence of C. zeae-maydis.

Using a DNA isolation kit (UltraClean™ Soil DNA Isolation Kit, Mo Bio Laboratories, Inc), total DNA was extracted from chaff/debris among the grains. The DNA samples were analysed by denaturing gradient gel electrophoresis (DGGE) after PCR amplification using of *C. zeae-maydis*-specific primers CZM2F + CZM2R (GENO§YS, UK) and universal fungal primers FR1-GC + FF390 (GENO§YS, UK). Fingerprint patterns produced by the DNA from the maize grain samples were subsequently compared with those of DNA extracted from pure cultures of *C. zeae-maydis.*

Crop debris

A series of experiments was conducted to investigate the microbial decay of infected maize leaves in soil. Leaves with three different levels GLS severity (nil, 'low', 'medium' and 'high') were buried in the soil at three depths (0cm, 10cm and 40cm) in between maize-growing seasons. The methods used in each country varied slightly. In Kenya, soil from a weed-fallow field with no history of maize cultivation was dug to a depth of 30cm, mixed using a shovel and all visible plant debris removed. The homogenised soil was put in perforated polythene bags measuring 15cm in diameter by 45cm

high; this constituted the working soil microcosm. In Zimbabwe, the soil was either sterilized or non-sterilized, placed in large pots and the crop debris was put into small muslin bags prior to burial.

For each level of disease severity, two leaf portions were placed at each depth. Destructive sampling was undertaken, at intervals of three weeks after burying, together with a control which was sampled on day 0. The experiment was laid out in a split-plot design with four replicates. Two leaf samples were buried for each treatment combination. Data recorded over time included the weight of maize leaves recovered; soil moisture and spores of *C. zeae-maydis* visible by microscopy. The inoculum load of *C. zeae-maydis* was determined on one of the two leaf portions by cutting the leaf into small pieces and agitating them in 100ml of a 0.01% Tween-20 solution in distilled water. The resulting suspension was then transferred, by decanting, into a clean beaker and the spore concentration estimated using a haemocytometer. This analysis took the form of a comparison between microbial observations of *C. zeae-maydis* spores against a soil background, with appropriate controls to allow for obscuring of spores by soil particles and plant material. Data arising from the assessment undertaken from August to September is reported here but samples from the second assessment i.e. March – April 2003 are currently being processed and will be reported elsewhere (Kinyua, 2003). The second leaf, and its adhering soil, was stored at –20^{o}C until in preparation for DNA extraction and analysis using molecular tools (Activity 3.3).

In Zimbabwe, a subsequent field experiment on crop debris was undertaken at Henderson Research Station in October 2001. Infected leaf blades weighing 10g were placed in vegetable packing material. Each 'pack' was placed in a hole 1.0m x 0.5m at one of three different depths of burial (10cm, 20cm and 30cm), and there were three replicates. Sampling was carried out every two weeks when the sample leaf blades at each depth were dug up, taken to the laboratory and examined microscopically for the presence of spores of *C. zeae-maydis*.

3.2 Epidemiological studies to identify factors influencing disease spread.

Four large-scale field trials were undertaken in Kenya (KARI, Kakamega) and Zimbabwe (PPRI, Henderson Research Station) over two consecutive growing seasons to identify factors influencing disease spread. Factors included in the experiments were selected on the basis of existing knowledge and data on GLS, together with new knowledge from the biological and socio-economic surveys (Activities 1.1 and 2.1).

- *Field Trial 1: Effect of soil fertility on the incidence and severity of maize GLS and yield in early, medium and late maturing maize hybrids.*
- *Field Trial 2: Effect of soil fertility on the incidence and severity of maize GLS and yield in early maturing maize hybrids grown in two contrasting levels of GLS-inoculum (low and high)*
- *Field Trial 3: Effect of 'type of maize maturity' (very early, early and medium maturing maize hybrids) fertility on the incidence and severity of maize GLS and yield under two contrasting levels of GLS-inoculum (low and high)*
- *Field Trial 4: Effect of fungicide on the incidence and severity of maize GLS and yield in early maturing maize hybrids grown in two contrasting levels of GLS-inoculum (low and high)*

Field Trial 1 was carried out at KARI-Kakamega to investigate the effect of two elements, Phosphorus (P) and Nitrogen (N) on the incidence and severity of GLS. The treatments included a low phosphorus site, a low nitrogen site and an 'optimum' site. The low phosphorus site had been developed by CIAT under a previous project i.e. the 'Bean Improvement for Low Fertility in Africa' project. Depletion of the field started in 1991 by intensive cropping without the application of phosphorus, and at the start of the experiment, an analysis of the soil using the Foster method indicated 0.81 ppm of phosphorus. The low nitrogen site was developed by continually growing maize at the site without the use of nitrogen-fertilisers. At the start of the trial, the level of nitrogen in the top soil was 0.22% and in the sub-soil was 0.16%.

The trial was set up during the long rains of 2001, and again in 2002. Eight varieties were used, three early maturing (H511, H513 and P3253), three medium maturing (H622, H623 and X1399AW) and two late maturing (H614 and H627). The trial design was a randomised complete block design with three replicates. At the low phosphorus-site, nitrogen was applied in the form of urea at the

recommended levels (60 Kg/ha) but no phosphate fertilizer was applied, while on the low nitrogen site, phosphorus was applied in triple phosphate form at planting at 60Kg phosphorus/ha and no nitrogen fertilizer applied. On the third site, that was considered an 'optimum' field (where there was no extraction of nitrogen and phosphorus), phosphorus and nitrogen fertilisers were applied at recommended levels. Planting was done as per the recommendations for the region with an inter-row spacing of 75 cm and intra-row spacing of 30 cm. Data recorded included the incidence and severity of GLS together with yield data.

Field Trials 2-4 were carried out at Henderson Research Station, Zimbabwe, in two contrasting fields – a 'low inoculum' field and a 'high inoculum' field. The 'low inoculum' field had not been planted with maize for the three previous growing seasons, did not have GLS infested maize debris on the field, no infested stover inoculum, and was situated upwind of the 'high inoculum' field. On adjacent fields, alternative crops (which were not known to host the GLS pathogen) or varieties of maize with known resistance to GLS were planted to act as barrier crops against any wind-spread inoculum. In contrast, the 'high inoculum' field was planted with a highly susceptible variety of maize in the previous growing season, were covered with GLS-infested maize debris, and after planting, GLS-infested stover was placed between the maize plants. In addition, susceptible varieties of maize were planted in the fields adjacent to the 'high inoculum' field.

In Zimbabwe, the seed company, SeedCo has the largest share of the maize seed market (>80%), so most of the maize hybrids used in the field trials were SeedCo hybrids, including very early (400 series), early (500 series) and medium (600 series) maturing hybrids. 700 series hybrids, which have a very long season, are grown exclusively by the large-scale commercial sector and were, therefore, not included in these field trials. Within each series, 400, 500 and 600, both GLS-tolerant and susceptible hybrids, which are often preferred by small-scale farmers, were compared.

Following land preparation i.e. ploughing, ridging, fertiliser application and laying out plot designs, the field trials were established. In Field Trial 2, the treatments were tolerant (0) and susceptible (1) hybrids of early maturing maize planted using three different fertilizer treatments; farmyard manure (F), recommended fertilizer rates (R) and a 50% increase in the recommended fertiliser rates (S). For each seed treatment, 675 seeds were used and there were three replicates, laid out in a randomised complete block design (Figure 3), with 18 plots (Figure 4).

For Field Trial 3, the treatments were tolerant (0) and susceptible hybrids (1) of very early (V), early (E) and medium (M) maturing hybrids (i.e. 400, 500 and 600 series) planted into 'low inoculum' and 'high inoculum' fields. Each seed treatment required 225 seeds and the experimental design and plot layout were as before (Figures 3 and 4).

In Field Trial 4, the treatments were tolerant (0) and susceptible (1) hybrids of early maturing maize grown in the absence of fungicide (F-) or with fungicide (Punch extra® applied according to manufacturers recommendations) (F+). Each seed treatment required 600 seeds, and there were four replicates. As there were only four treatment combinations, this field trial required only 16 plots.

For all field trials, data for soil fertility was collected prior to the start of the trials and after harvest. Incidence and severity of GLS was assessed weekly on the 'net plot' i.e. 30 sample plants per plot from the date of planting onwards. Although GLS does not usually appear before tassling, the exact date of initial symptom expression needed to be confirmed. Once symptoms had appeared, GLS was scored weekly. Other variables recorded included root lodging, stem/stalk lodging and harvest date, together with the yield at harvest.

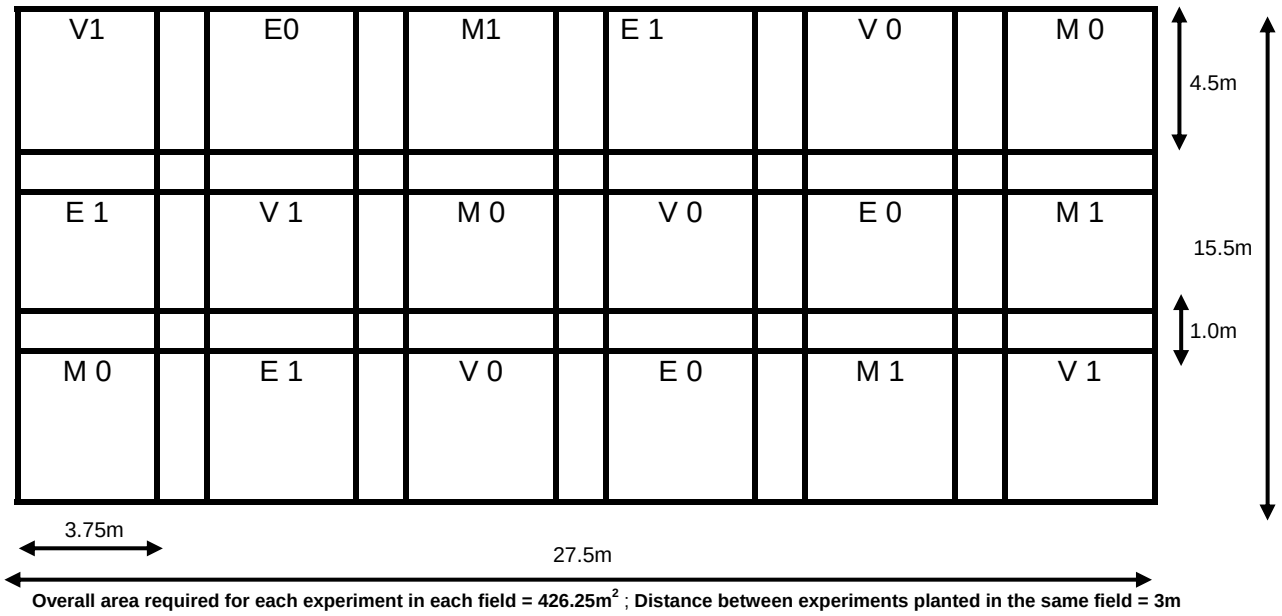


Figure 3: Diagram showing the experimental design used for Field Trials 2, 3 and 4, carried out at Henderson Research Station, Zimbabwe in 2001/02 and 2002/03 maize growing seasons.

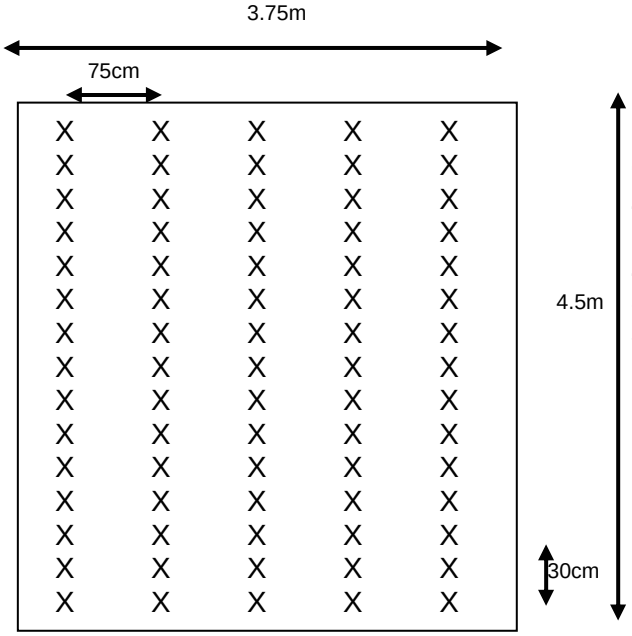


Figure 4: Diagram showing Plot dimensions and layout for Field Trials 2, 3 and 4 which were carried out at Henderson Research Station during the 2001/02 and 2002/03 maize growing seasons. X – single maize plant, 5 - rows per plot and 15 - maize plants per row.

3.3 Molecular markers used to track spread of individuals from inoculum sources

The combination of low genetic diversity amongst the populations of *C. zeae-maydis* and the failure to identify any alternate host of *C. zeae-maydis* meant that it was not possible to confirm the epidemiology of GLS using molecular markers.

Use of molecular tools for the detection of *C. zeae-maydis* during decay of plant debris

Data arising from an earlier experiment on crop debris (see Activity 3.1) placed emphasis on the need to understand the fate of inoculum of *C. zeae-maydis* in plant debris and soil between growing seasons. DNA was extracted from the second leaf sample using the UltraClean™ Soil DNA Isolation Kit (Mo Bio Laboratories, Inc) and subjected to analyses for total bacterial and fungal activity in addition to detection of *C. zeae-maydis*. Universal bacterial primers UNIBACT3GC (GENO§YS, UK) + UNIBACT2 (amersham pharmacia biotech), universal fungal primers FR1-GC + FF390 (GENO§YS, UK) and *C. zeae-maydis*-specific primers CZM2F + CZM2R (GENO§YS, UK) were used for amplification in the respective analyses before carrying out the denaturing gradient gel electrophoresis (DGGE). This approach was facilitated by the assistance of Dr Larry Dunkle of

Purdue University, USA, who provided information on purportedly Group 2 selective primers [forward: 5'-GCG ACC CTG CCG TTT and reverse: 5'-CTC AGC CGG AGA CTT CG].

Data recorded over time included the quantity of total DNA extracted and presence/ absence of DNA of *C. zeae-maydis* (PCR specific product). The quantities of total DNA obtained from maize debris/soil samples were measured using a GeneQuant Pro RNA/DNA Calculator (Biochrom Ltd., England). Results from these analyses are currently being matched with the available visual assessment data, and will be used to determine the survival of *C. zeae-maydis* inoculum under the test conditions (Kinyua, 2003).

Linked to this assessment further analyses were taken on the total soil DNA extracts to investigate background population dynamics of other microbial populations. These studies were considered speculative, but aimed to identify key microbial communities that promoted plant material decay. The approach taken was based on PCR analysis of the conserved ITS regions using GC clamped primers and DGGE of total DNA extracts. Preliminary analyses using this approach with eubacteria conserved GC clamped primers gave complex profiles by DGGE. These profiles were seen to change over time in relation to the decay status of the material, but the complexity and heterogeneity of these profiles makes it unlikely that these data can be analysed further to show any systematic shifts in population structure.

4.1 Establish and validate standard protocol for screening for resistance

A review of current literature on maize GLS indicates a range of different protocols are being used to screen for resistance to maize GLS around the world. These include the use of artificial or natural inoculum, of one or more isolates of *C. zeae-maydis* on individual leaves, part or whole maize plants in glasshouse or field trials. Typically, GLS is assessed at several key growth stages, or at a specified number of days after planting, from the time of silking/tassling onwards. Existing methods of scoring for GLS include quantitative methods such percentage leaf area infected (Clements *et al.,* 2000), measurements of lesion expansion (Ajanga *et al.,* 2001) or the use of standard assessment diagrams (Smith, 1989), and subsequently modified by Ward *et al.* (1996, 1997b). Subjective severity scores for GLS include 1=Excellent, 2=Very good, 3=Good, 4=Fairly good, 5=Average, 6=Slightly susceptible, 7=Moderately susceptible, 8=Susceptible, and 9=Very susceptible (Seed Co, 2000), or a rating using a 0.5-5 scale in increments of 0.5 where 0.5 = a few restricted lesions on lower leaves, 1.0 = several scattered lesions on lower leaves, 2.0 = several lesions on lower leaves, with a few on middle leaves, 3.0 = several lesions on middle leaves with abundant lesions on lower leaves, 4.0 = several lesions on upper leaves with abundant lesions on middle and lower leaves, 5.0 = abundant on all leaves.

Existing protocols for screening for resistance to GLS were evaluated and compared for their accuracy, efficiency, degree of complexity and cost. Based on this information, a simplified, rapid protocol was developed using a quantitative scoring system i.e. percentage leaf area infected on a whole plant, but with only five categories of severity (including zero). The simplified, rapid screening protocol was then tested alongside existing methods of screening for resistance in field trials in Kenya and Zimbabwe.

4.2 Screen available maize germplasm against representative cultures of pathogen isolates in Kenya and Zimbabwe

In Field Trial 5, twelve varieties of maize which are commercially available in Kenya i.e. H614, H622, H623, H625, H626, H627, H628, H512, P3253, C4141, KSTP94, and H513 were evaluated for their tolerance/resistance to GLS during the long-rain seasons of 2001 and 2002. The trials were carried out at KARI-Kakamega station, a known 'hot spot' for GLS, using a randomised complete block design with four replicates. Each plot consisted of 5 rows of 20 plants, although only the inner three rows were sampled. The planting was as per the recommended spacing and fertilizer applications. Infected stovers of maize variety H623, saved from the previous season, were uniformly laid between the rows to serve as an additional source of inoculum after planting i.e. 'enhanced natural inoculum'. Data for the incidence and severity of GLS were recorded weekly, together with the yield at harvest.

A similar field trial (Field Trial 6) was carried in Zimbabwe at the Agricultural Research Trust Farm of the Commercial Farmers Union, Zimbabwe in 2002 and 2003. Each year, the four main seed companies in Zimbabwe, i.e. SeedCo, Pioneer, Pannar and Monsanto supply seed for comparative performance trials at ART farm. ART farm also happens to be a disease hotspot for GLS. The trial was laid out in varietal blocks, each consisting of four plots each with five rows and 20 maize plants per row. CFU kindly allowed scientists from the project to make regular assessments of the varietal trials in order to screen available maize germplasm for the incidence and severity of GLS, together with yield.

4.3 Identify suitable disease resistance screening sites and suitable isolates for germplasm screening programmes

Data from the nationwide biological surveys for GLS in Kenya and Zimbabwe were used to identify areas which would be suitable for disease resistance screening (see Figures 5 and 6). As for Activity 4.1, the similar, if not identical, pathotypic lineages, exhibited by isolates of *C. zeae-maydis* in both Kenya and Zimbabwe (Activity 1.4), means that there is little or no difference in the pathogenicity of isolates of *C. zeae-maydis,* which precludes the need to identify suitable isolates for germplasm screening programmes, and as such, there is no justification for increasing the number of isolates used in resistance screening programmes.

4.4 Establish screening technologies into national maize breeding/selection programmes

In both Kenya and Zimbabwe, the National Programmes had developed screening technologies for maize GLS and incorporated them into their maize breeding/selection programme by the time the current project was initiated. CIMMYT, which has regional offices in Nairobi, Kenya and Harare, Zimbabwe also includes screening for maize GLS in its characterisation of maize germplasm grown in Eastern and Southern Africa. CIMMYT's breeding/selection programmes are carried out in close collaboration with the National Programmes, particularly in Kenya. Similarly, the commercial maize seed companies in Zimbabwe, have been screening their maize germplasm for GLS since 1997, and more recently, by commercial seed companies in Kenya. During the course of this project, maize breeders from the public and private sector in Kenya and Zimbabwe were visited to discuss current screening technologies for GLS. Given that screening technologies were already established within national maize breeding/selection programmes, emphasis was given to improving and standardising GLS screening technologies.

5.1 Develop preliminary recommendations for an IPM strategy

On the basis of findings from the socio-economic survey (Activities 2.1 & 2.2) and the epidemiological studies (Activities 3.1 & 3.2), preliminary recommendations for the management of maize GLS by small-scale maize farmers were formulated. Following the findings of the socio-economic survey, conducted at the start of the project, it was apparent that awareness of GLS and the potential yield losses associated with the disease amongst small-scale maize farmers and extensionists continues to be surprisingly low. An essential pre-requisite to any management strategy is the ability to recognise the problem! To this end, a 'Raising awareness of maize GLS' poster was produced in Swahili, Shona and English versions and disseminated to >5000 small-scale farmers and extensionists as appropriate in Kenya and Zimbabwe (see Appendix 5).

The preliminary IPM strategy was based on a 'basket of options' including crop rotation, management of crop debris and soil fertility, together with the use of resistant varieties. The preliminary strategy was refined following feedback from CIMMYT, farmer groups, extensionists, seed companies and researchers in Kenya, Zimbabwe and South Africa. A leaflet on 'Options for managing maize grey leaf' was subsequently prepared in Swahili, Shona and English versions and disseminated to 'pilot' small-scale farmers and extentionists. It is intended that the leaflet is disseminated in conjunction with training as appropriate i.e. as a component of a 'training of trainers,' or farmer-participatory training (see Appendix 6), and to date, >1500 leaflets have been disseminated.

5.2 Facilitate collaborative farmer-participatory experimentation to validate the strategy

The IPM strategy was validated in two contrasting districts in Kenya – Kiambu where the severity of GLS is low and Kakamega which is a GLS 'hotspot'. Prevailing conditions meant that it was not possible to validate the strategy in Zimbabwe although the 'Raising awareness of maize GLS' posters, together with the leaflets on 'Options for managing maize grey leaf' in both Shona and English have been disseminated to seed companies, extentionist and researchers working on Maize in Zimbabwe. A training exercise on GLS (awareness raising and management options was conducted at the District Agricultural Office with 25 extension staff representing all seven divisions of Kiambu District. At the beginning of the exercise, none of the participants was aware of GLS but following an initial 'probing' session and subsequent distribution of the poster and leaflets, ten of the extensionists recognized the symptoms but had not been aware of the name of the disease. Following the training session for extensionists, field days for farmers were hled in each of the 7 divisions represented by the extensionists. As the field days coincided with the beginning of the planting season, attendance at all of the field days was high i.e. >40 small-scale maize farmers at each of the field days, and in total, more than 300 farmers in Kiambu were involved in validating the IPM strategy. During the field days, a farmer-participatory session of GLS was facilitated by Mr Martin Kimani, Farmer Participatory Training & Research, Specialist, CABI-ARC. None of the participating farmers had seen GLS before, even though GLS was present on some of the 'host' farms, albeit at a low incidence. During the session, farmers collected leaf and maize cob samples and used the posters to identify GLS. Appreciation of the participatory training sessions together with the poster and leaflets was unanimous, with 100% of farmers claiming that they would now be able to recognize GLS in their shambas. At present, the levels of GLS in Kiambu do not warrant management strategies although farmers are aware of potential management options for future use.

Plate 4: A 'training of trainers' session on GLS

Plate 5: A field day on GLS for a womens group, Kiambu

Plate 6: Participatory approaches used to describe GLS

Plate 7: Training on GLS at a FFS in Kakamega

In Kakamega, the approach was slightly different in that the training sessions on GLS were facilitated during the regular weekly Farmer Field School sessions which are co-ordinated by the Global IPM Facility. In this way, training exercises on GLS simultaneously involved the farmers and the extensionists (trainers). The sessions were again facilitated by Mr Martin Kimani using the same approach as for Kiambu. 40% of participating farmers had seen GLS and some of the farmers had observed severe levels of the disease on their farms. Once again, farmers were unanimous in their appreciation of the training session, poster and leaflet. Farmers who had experience of severe levels of GLS (20%) were relieved to learn that there were options available for managing the disease which they now intend to implement in future maize crops (Plates 4-7).

Outputs

Key achievements and outputs of the project are summarised (below), with the 'cross-cutting' outputs appearing first, followed by the achievements under the specific project outputs, as per the project log-frame.

- ***Two successful stakeholders' workshops on maize GLS organised and hosted in Kenya and Zimbabwe***

Plate 8: Participants at a Stakeholders' workshop on GLS, Harare, March 2003

During the three-year project, the project team organised and hosted two successful stakeholders' workshops on maize GLS. The first, 'project-launching workshop' was held from 12-13th October 2000 at CABI-ARC, Kenya with 23 participants from four countries (Simons, 2000). The second workshop entitled, 'Maize grey leaf spot: a problem solved?' involved 35 participants from five countries (Plate 8), and was held 6-7th March 2003, Harare, Zimbabwe (Simons, 2003).

- ***Four training attachments in the molecular characterisation of isolates of GLS organised and hosted at CABI-UKC***

Extensive training in the molecular characterisation of isolates of GLS was undertaken by two of the project scientists, Mr Z.M. Kinyua, KARI and Mrs E. Mtisi, PPRI, in four separate attachments with Dr Julian Smith, Molecular Plant Pathologist at CABI-UKC. The attachments were undertaken by Mr Z. Kinyua and Mrs E. Mtisi, April-June 2001 (Kinyua, 2001; Mtisi, 2001), Mr Z. Kinyua, September-December 2002 (Kinyua, 2002) and Mr Z. Kinyua, June-August 2003.

- ***Post-graduate training (PhD) in the 'Genetic structure and virulence characterisations of* Cercospora *populations causing maize grey leaf spot in Kenya'***

Under the auspices of the current project, Mr Z. Kinyua was able to pursue his PhD studies with research costs and fees funded by the project. The title of his thesis was, 'Genetic structure and virulence characterisations of *Cercospora* populations causing maize grey leaf spot in Kenya'. Although there was no financial provision for this output in the original proposal, it was made possible because of a unique agreement between CABI-UKC and Royal Holloway, University of London, which provides for a large reduction in fees where studies are undertaken at CAB *International*, and additional 'study support' provided by KARI. Mr Z. Kinyua is expected to defend his PhD thesis in October 2003 (Kinyua, 2003).

Output 1: Variability of pathogen population determined using representative isolates from both Kenya and Zimbabwe.

- ***Distribution and severity of maize GLS on small-scale farms in Kenya and Zimbabwe quantified***

The distribution and severity of GLS on small-scale maize farms in Kenya and Zimbabwe has been quantified and mapped for the first time, using data collected during nationwide biological surveys (see Figures 5 and 6). In Kenya, GLS was found to occur in all of the maize producing regions, albeit at a low severity, only 7 years after it was first observed, which indicates a rapid rate of disease spread. Some areas already have severity scores of >50%! Of the 250 farms assessed, 74% had GLS in Kenya. Five districts in Western Kenya (Kisii, Bomet, Migori, Rachuonyo and Kakamega), together with one district (Taita Taveta) in Coastal Province were considered to be disease 'hot spots' in Kenya.

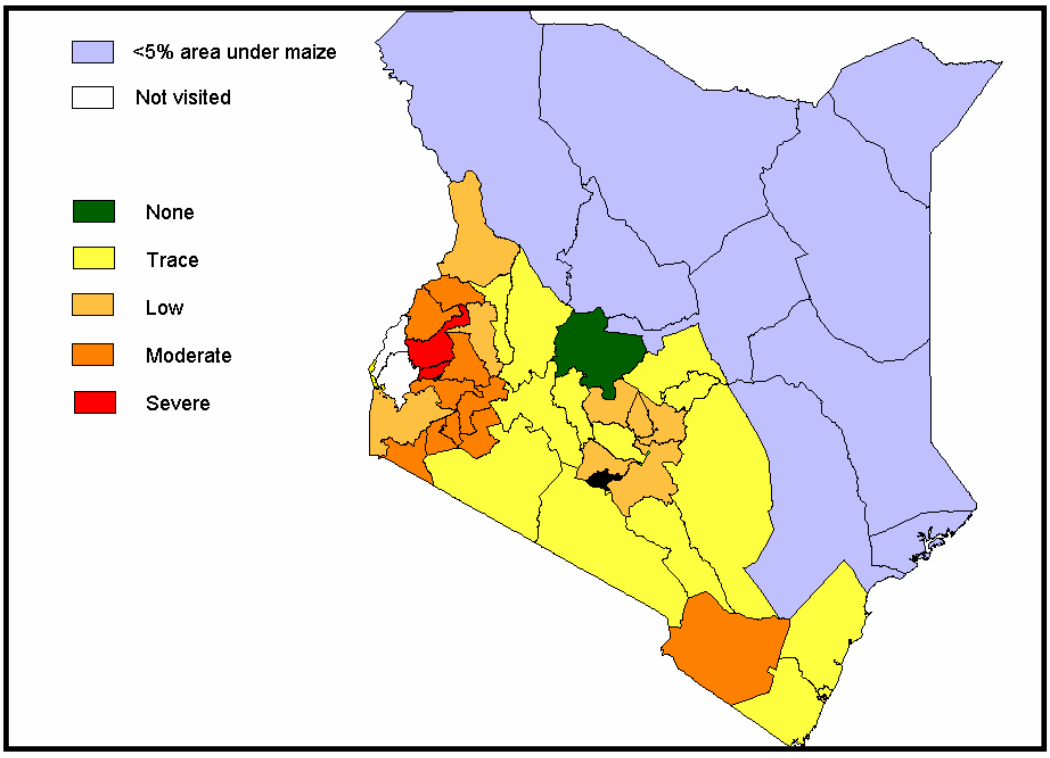


Figure 5: A map showing distribution and severity of GLS in Kenya

In Zimbabwe, GLS was found on 98% of small-scale maize farms and in all areas surveyed. Severity levels have shown some association with region. The percentage of farmers in region III found to have severe GLS symptoms on their maize was lower than expected, when compared to the other main maize growing regions IIA and IIB This may be linked to the lower rainfall in the marginal maize growing region III. The disease 'hot spots' were primarily, although not exclusively in and around Harare, including the districts of Harare, Mutasa, Macheke, Chinamhora, Mazowe, Wedza, Selous, Marondera, Hurungwe, Masvingo and Chipinge.

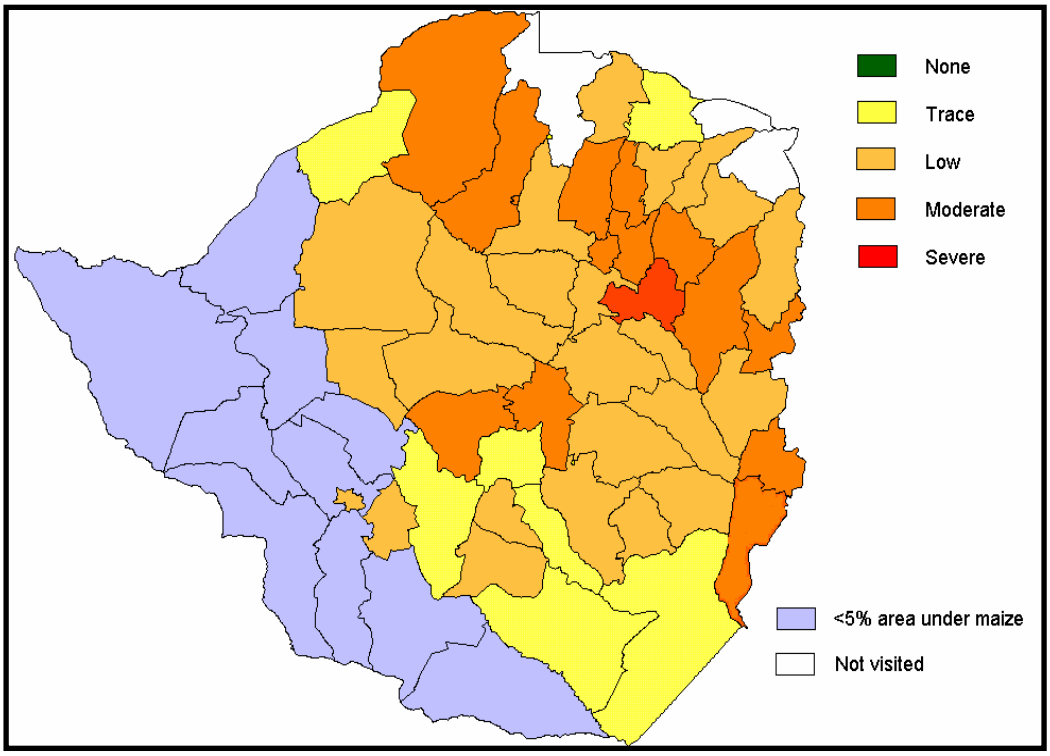


Figure 6: A map showing distribution and severity of GLS in Zimbabwe

- ***A total of 372 isolates of* Cercospora *spp. were single spored and established in culture***

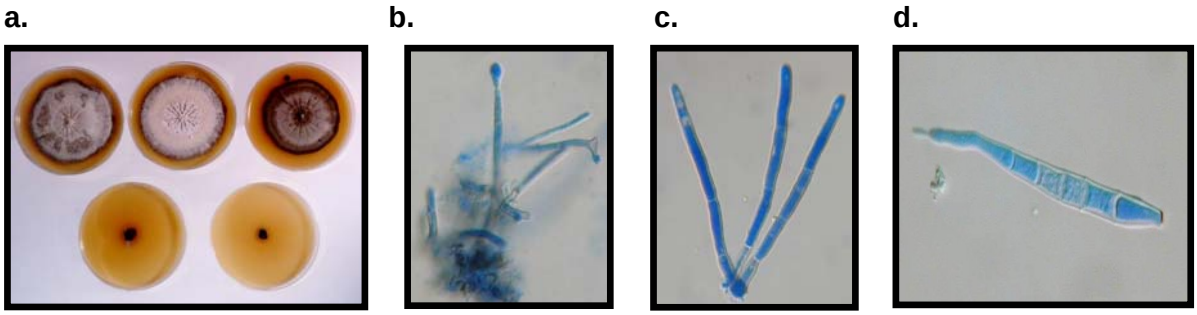


Plate 9: a. Typical 26-day cultures of *Cercospora sorghi* (top three cultures) and *Cercospora zeae-maydis* (lower two cultures) on V8 agar; b. Conidiophore and conidiogenesis in *C. zeae-maydis*; c. Synnema in *C. zeae-maydis*; d. mature conidium of *C. zeae-maydis* (Courtesy of Mr Z.M. Kinyua).

- ***Culture collection of species of* Cercospora *spp. established and maintained***

A combined culture collection with 250 representative isolates of *Cercospora* spp. has been established. 196 isolates have been taxonomically identified as *C. zeae-maydis* (141 from Kenya and 57 from Zimbabwe) and 14 as *C. sorghi* (5 from Kenya and 7 from Zimbabwe). The Kenyan isolate collection is housed at KARI & CABI-ARC and the Zimbabwean isolate collection is housed at PPRI, with a duplicate sub-set housed at CABI-UKC. Also maintained in the collection at CABI-UKC is a further 25 isolates of *C. zeae-maydis* from other countries, together with other species of *Cercospora* i.e. *C. beticola*, *C. longipes* and *C. sorghi*.

- ***Studies using ITS-RFLP and AFLP confirmed the existence and delineations of two species of* Cercospora *causing GLS i.e.* C. zeae-maydis *(95%) and* C. sorghi *(5%).***

Both ITS-RFLP and AFLP analyses easily distinguished *C. zeae-maydis from C. sorghi* and other species of *Cercospora* e.g. *C. longipes*. It was interesting to note that *C. beticola* was closely related to both *C. zeae-maydis* and *C. sorghi* var *maydis* groups. RFLP analysis has detected 12 isolates of *C. sorghi* to date, all from areas in Kenya and Zimbabwe with a high incidence of GLS (see Figure 7).

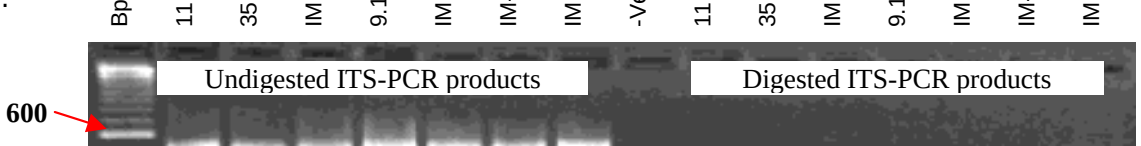


Figure 7: Comparison of fungal isolates of different species of *Cercospora* species after Taq*I* digestion of ITS-PCR products. Czm - *C. zeae-maydis*, Cs - *C. sorghi*, Cb - *C. beticola* and Cl - *C. longipes.* IMI indicates that the isolate is from CABI's Microbial Genetic Culture Collection.

- ***Isolates of C. zeae-maydis from Kenya and Zimbabwe were all found to belong to* Cercospora zeae-maydis *Group II.***

Analysis of the ITS-RFLP product revealed that isolates of *C. zeae-maydis* from Kenya, Zimbabwe and South Africa were all strongly similar to *C. zeae-maydis* group II, as described by Wang *et al.* (1998) in the USA and they were clearly separated by large genetic distances from isolates of *C. zeae-maydis* group I. No group I isolates of *C. zeae-maydis* were recovered from the biological surveys in either Kenya or Zimbabwe. This finding concurs with analyses of isolates of *C. zeae-maydis* from other African countries including South Africa, Zimbabwe, Zambia and Uganda (Dunkle and Levy, 2000). The representative isolates of *C. zeae-maydis* groups I and II from the USA used in these studies were kindly provided by Prof. Larry Dunkle of Purdue University, USA.

Previous studies have speculated on the recent origin of *C. zeae-maydis* in Africa. The current majority opinion centres on an introduction through grain shipments from the USA, where both Groups I and II of *C. zeae-maydis* have been reported. The data obtained in this study, that reports on the most extensive population of African isolates analysed to date, are consistent with these previous studies, recording only Group 2 present in East Africa, but do not provide any more substantive evidence as to the origins of the pathogen.

- ***AFLP analysis showed the population of* C. zeae-maydis *to be highly homogeneous (>98%)***

Analysis of the AFLP fingerprints, revealed a high degree of homogeneity (>98%) amongst the isolates of *C. zeae-maydis*, with only very minor genetic variation (<2%) being recorded, irrespective of the primers used (see Figures 8 and 9). This finding is in agreement with reports from elsewhere in the world (Dunkle and Levy, 2000; Stromberg *et al.,* 2000; Wang *et al.,* 1998). Such clustering has in other pathogens frequently been related to geographic and/or host interactions. In this case, a limited number of polymorphic loci could be detected among the isolates of *C. zeae-maydis,* however, analysis of isolates from different districts showed that this diversity was not region specific. The limited variation is important in breeding/selection for resistance to GLS because materials showing true resistance/tolerance to the disease in one location can also be used over a wide range of environments, provided that the maize hybrid/variety is adapted to those conditions. In the current study however, the apparently low levels of genetic diversity recorded, also meant that meaningful analyses of potential sub-population structures was not possible.

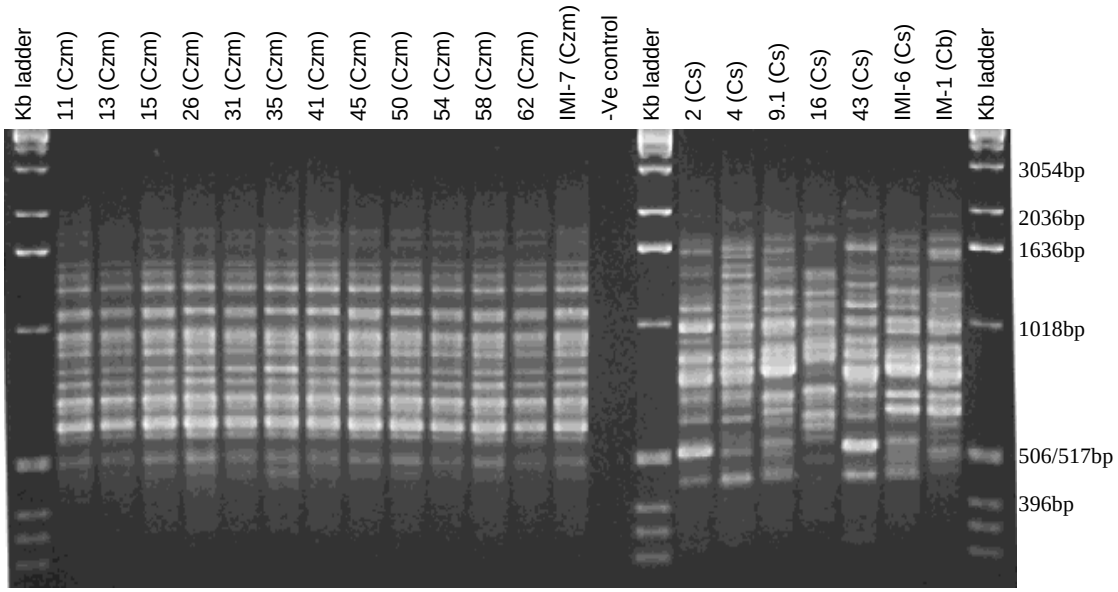


Figure 8: AFLP fingerprint patterns generated on representative isolates of *Cercospora zeae-maydis* from different regions of Kenya, together with known species of *Cercospora.* Czm - *C. zeae-maydis*, Cs - *C. sorghi*, Cb - *C. beticola* and Cl - *C. longipes.* IMI indicates that the isolate is from CABI's Microbial Genetic Culture Collection.

- ***AFLP analysis showed the population of* C. sorghi *was highly heterogeneous (84-88%).***

In contrast to *C. zeae-maydis*, isolates of *C. sorghi* var *maydis* were highly divergent, irrespective of the primers used, with genetic similarities ranging from 84 to 88%. Indeed, the level of diversity recorded between these isolates was comparable to that observed between the outlying near-relative *Cercospora* species used for comparative purposes in the study (see Figures 8 and 9).

While *C. zeae-maydis* was found in all maize-growing areas where GLS was recorded, *C. sorghi* was only isolated from the Western Region of Kenya and Harare Region of Zimbabwe. It was, however, a significant finding to observe *C. sorghi* as a minor pathogen on maize. It was also significant that *C. sorghi* and not *C. zeae-maydis* was isolated from GLS-like symptoms on various potential alternative hosts (Activity 3.1). These contrasting observations between *C. zeae-maydis* and *C. sorghi,* of a founder and long established pathogen and a pathogen of narrow and broad host range, were evident in the AFLP analyses. It can be speculated that both time for adaptation and host range boundaries has provided, in part, the drivers for genetic variation evident in these species. In this context, the apparently low genetic diversity recorded amongst the isolates of *C. zeae-maydis* was consistent with both a founder population and a pathogen with a very strong dependency on a single host. Whereas the high genetic diversity amongst the *C. sorghi* isolates was consistent with a long-established pathogen that possessed the genetic plasticity to exploit a range of host genotypes.

- ***Little or no evidence for the existence of pathotypic lineages***

There were no discernable differences in the rate of lesion development between the isolates of *C. zeae-maydis* on detached leaves although rate of lesion development from isolates of *C. sorghi* was significantly slower in both Kenya and Zimbabwe ($p<0.01$). The two differential isolates of *C. zeae-maydis* also reacted in a similar manner on individual maize hybrids in terms of number and length of GLS lesions (Figure 10). Analysis of the data recorded at the final disease assessment (120 days after planting), excluding control treatments, revealed no significant difference between the isolates for number of lesions ($P>0.912$), dimensions of lesions ($P>0.926$) or yield ($P=0.551$), whereas the difference between hybrids for the same parameters was highly significant ($P<0.03$; $P<0.001$ and $P<0.001$ respectively). There was no interaction between isolate and variety.

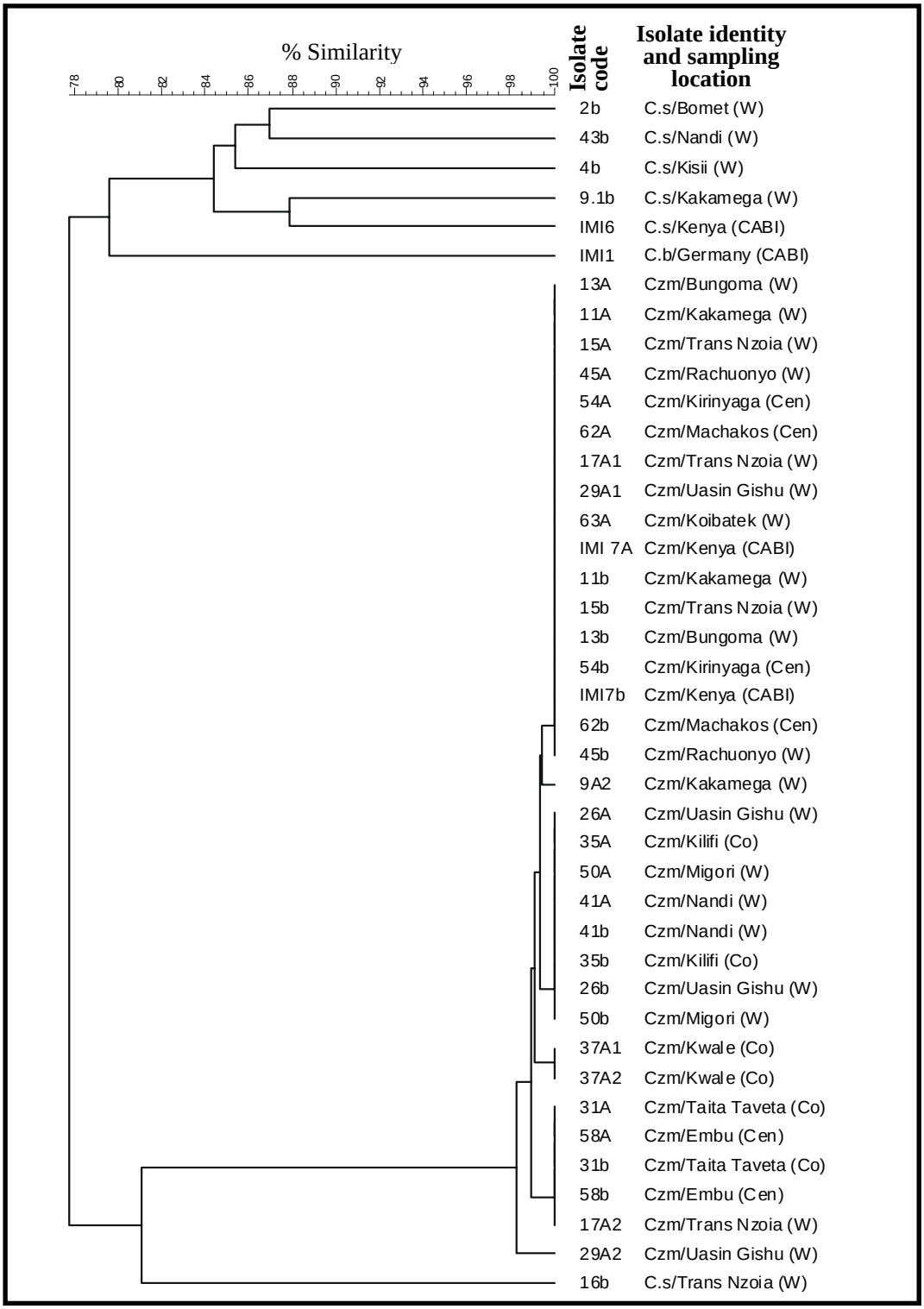


Figure 9: Phylogenetic tree of AFLP fingerprints from representative isolates of *Cercospora zeae-maydis* and *C. sorghi* from maize in Kenya, together with other species of *Cercospora* from CABI's Microbial Genetic Resource Collection. Czm=*C. zeae-maydis*, Cs=*C. sorghi* and Cb *C. beticola.*

This outcome of the pathogenicity studies i.e. the absence of discernable variation, was consistent with the apparent lack of genetic diversity amongst the isolates (Activity 1.3), which was in turn reflective of the nationwide position in both countries. The minor genetic difference detected between the two Kenyan isolates through AFLP analysis (Kinyua, 2001), was not reflected through GLS symptom expression. This finding is in agreement with that of Lipps *et al.* (1998), who also found that molecular-based differences between two populations of *C. zeae-maydis* in the USA, were not transformed into differences in virulence or infection phenotype. In the current study, therefore, it was not possible to assess the extent of evidence for pathotypic lineages that might be used in future resistance screening programmes (Activity 4.1). To confirm this assertion, in subsequent studies, varietal/strain interactions have been assessed using isolates from composite populations of *C.*

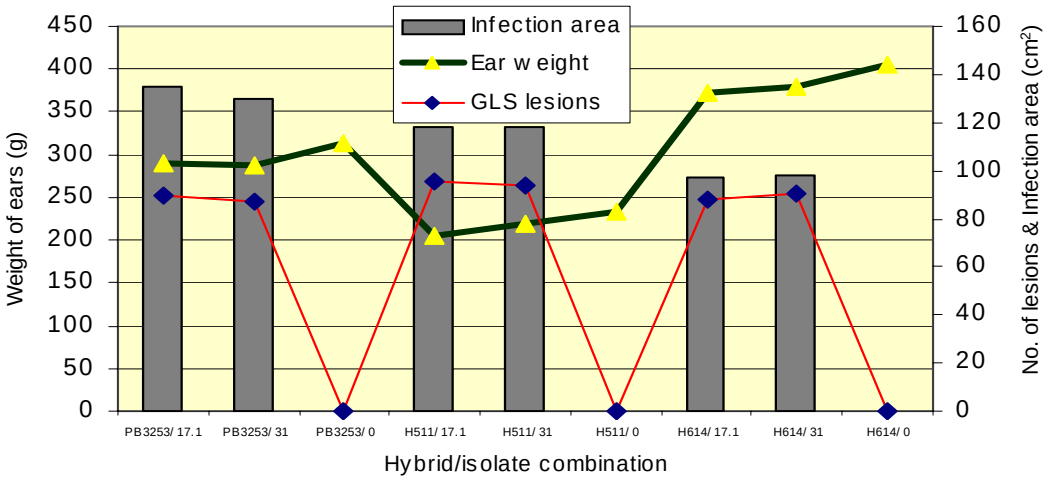


Figure 10: Graph showing the effect of two contrasting isolates of *Cercospora zeae-maydis* on three maize hybrids (PB 3253, H511 and H614D)

zeae-maydis with a view towards tailoring a protocol for pathogenicity assessment appropriate to the rapid screening of germplasm, as might be required in a breeding programme. Results from these additional studies will be reported later this year (Kinyua, 2003).

Output 2: Cultural practices affecting disease incidence and severity identified.

- ***Successful completion of the first socio-economic survey of GLS on small-scale maize farms in Kenya and Zimbabwe (CABI-ARC, 2002)***

This was the first socio-economic survey of maize GLS undertaken in Africa. The survey was conducted on ~220 small-scale maize farms in Kenya and Zimbabwe in selected maize-growing areas where GLS was known to be prevalent (identified during the biological survey (Activity 1.1)) and covering a range of ecosystems. The survey provided comprehensive information on the farm management characteristics of small-scale maize farmers in GLS-prevalent areas, the effects of maize variety, crop management practices and post-harvest management practices on the incidence and severity of GLS, relationships between other insect pests and diseases affecting maize production and GLS, and farmers perceptions of GLS and the yield loss attributable to GLS (see CABI-ARC, 2002).

- ***Farm management characteristics of small-scale maize farms in GLS-prevalent areas in Kenya and Zimbabwe***

During the socio-economic survey, farmers were asked questions concerning farm management characteristics e.g. method of ploughing. A summary of farm management characteristics is as follows (full details can be found in CABI-ARC (2002)):

- In Western Kenya, most farmers' plant maize two crops, whereas in Zimbabwe, farmers plant just one crop of maize per year.
- Most farmers in Western Kenya (76%) do not rotate their maize crop whereas in Zimbabwe, 67% of farmers practice crop rotation.
- Where crop rotation is practised the main alternate crops in Kenya are vegetables (beans, onion, potato and sweet potato), millet, wheat or sunflowers, whereas in Zimbabwe, the main alternative crops are groundnuts and cotton.
- In both countries, even where crop rotation is practised by small-scale farmers, it is unusual for the entire maize crop to be rotated.
- Average farm size in the areas surveyed was 2.5 ha in Kenya and 6.5 ha in Zimbabwe.
- Small-scale farmers in both countries committed a significant percentage of their farms to maize production i.e. ~50% (48% in Kenya and 54% in Zimbabwe).
- Most farmers grow hybrid maize varieties (84% in Kenya for first crop, 53% for the second crop; and 94% in Zimbabwe) – the most popular varieties are H614 and H627 (Kenya) and SC 401, 501 and 513 (Zimbabwe).

- The majority of farmers ploughed by draught (Kenya 33%; Zimbabwe 84%), either alone or alongside tractor or hand ploughing.
- 85% and 56% of farmers weed their crop twice per season in Kenya and Zimbabwe respectively.
- In both countries, ~85% of farmers were using some form of inorganic fertilizer.
- A ranking of pests and diseases rated stem borers, followed by termites or cut worms as the most important insect pests, and Maize GLS and maize streak virus as the most important diseases in both countries.
- 41% of farmers were applying inorganic fertiliser as top dressing in Kenya compared with 83% in Zimbabwe.
- Of the farmers who were applying fertiliser, the majority admitted that they were applying less fertiliser, sometimes substantially less, than the recommended levels.
- 8% and 11% of farmers applied chemical insecticide to their maize in Kenya and Zimbabwe respectively
- In Kenya, the majority of farmers (51%) removed maize debris from the field after harvest whereas in Zimbabwe, 67% practised early ploughing.

- ***Clear evidence for the effect of maize variety on the incidence and severity of maize GLS***

During the socio-economic survey, farmers were asked questions concerning which maize variety (ies) they grew and the effect of maize variety on the incidence and severity of GLS. There was a clear relationship between maize variety and the incidence and severity of GLS. In Zimbabwe, 59% of farms had severe levels of GLS which corresponded directly with the use of susceptible varieties ($P<0.01$) - tolerant varieties showed only trace symptoms whereas susceptible varieties showed moderate to severe levels of GLS. This relationship was observed in all natural regions and independently of all crop and post-harvest management practices, and was particularly pronounced on farms where both GLS-tolerant and GLS-susceptible varieties of maize were grown. In Kenya, maize variety had a similar effect on GLS although the relationship was not as clear which probably reflects the lack of commercially available varieties of maize which have been bred for resistance to GLS - the disease occurs on hybrid, 'local' and open-pollinated varieties, and because the farmers tended to be less aware of the actual maize variety they were growing (or had been given inaccurate information when they purchased the seed). Moderate levels of GLS were observed on all 'local' maize varieties and on the open-pollinated variety, Katumani. Of the hybrids, GLS was observed at low levels on H513 and H625, moderate levels on H511, but at all levels of severity on H614 and H627. Farmers' perceptions supported by observations made by the research team were not always consistent with statements made by the maize seed producers concerning GLS tolerance. Full details can be found in CABI-ARC (2002).

- ***Little or no evidence for a correlation between cost of maize seed and GLS presence/severity.***

There was no obvious correlation between cost of maize seed and GLS incidence/severity in either country. Price information from the seed companies in Kenya indicates that there is no distinction between the cost of GLS-tolerant and susceptible maize varieties. Similar information from Zimbabwe also indicates that varieties of maize which were marketed as being GLS-tolerant did not cost significantly more than GLS-susceptible varieties. It should be noted, however, that when GLS-tolerant maize seed first came onto the market in Zimbabwe in 1997, they were more expensive and until 1999, most of the GLS-tolerant varieties were 'late maturing' varieties which tended not to be used by small-scale maize farmers. The situation in Zimbabwe has now changed with early and medium maturing GLS-tolerant varieties entering the market and all seed companies have reduced the price of their GLS-tolerant seed to make it more accessible to small-scale farmers. It is likely therefore that most farmers who are continuing to purchase susceptible varieties are doing so because of a lack of awareness of GLS rather than because they cannot afford to purchase tolerant varieties. However, some small-scale farmers expressed a clear reluctance to change their maize variety because they knew how their particular variety performed and what rate of maturity/yield to expect. In such cases, farmers had only changed their preferred variety of maize after losing a significant amount of their maize crop to GLS.

- ***Relationship between crop management practices and incidence/severity of GLS quantified***

Crop management practices found to be influencing the incidence and severity of maize GLS during the socio-economic survey were consistent with literature on the disease from other countries. Figure 11 shows the relationships between crop management practices and the incidence/severity of GLS in Zimbabwe and Kenya respectively. Chi-square tests were carried out to investigate whether the number of farms with severe GLS was related to crop management practices. In Zimbabwe, significant levels of association were found for 'number of weedings' ($p<0.001$) and mixed/intercropping' ($p=0.003$). Association for weeding practice implies that the occurrence of severe levels of GLS was lower than expected (if no association) on crops that were weeded only once and higher on maize weeded more than once. Additionally, the frequency of occurrence of severe GLS on monocropped maize was lower than expected and higher on maize planted alongside other crops. In Kenya, it was not possible to carry out similar tests to compare GLS presence (at all levels) with crop management practices, as expected values for the majority of observations were too low (<5). The percentage of farms with the Incidence/severity of GLS in relation to crop management practices is shown in Figure 11. Chi-squared tests to test for association between management practices and GLS incidence/severity proved invalid, due to the low numbers.

Overall, there were no clear effects of crop management practice on incidence/severity of GLS although there was some evidence to suggest that greater regularity of weeding was associated with higher levels of GLS. The absence of effects attributable to inorganic fertiliser may be due to the fact that farmers rarely applied the recommended levels and, in general, did not fertilise all of the land under maize. Researchers observed that on high fertility areas of the farms (e.g. anthills) the GLS levels were notably higher than where the soil suffered from low fertility (e.g. sandpits). These relationships are investigated in more detail in controlled field trials carried out in Zimbabwe and Kenya (see Output 3).

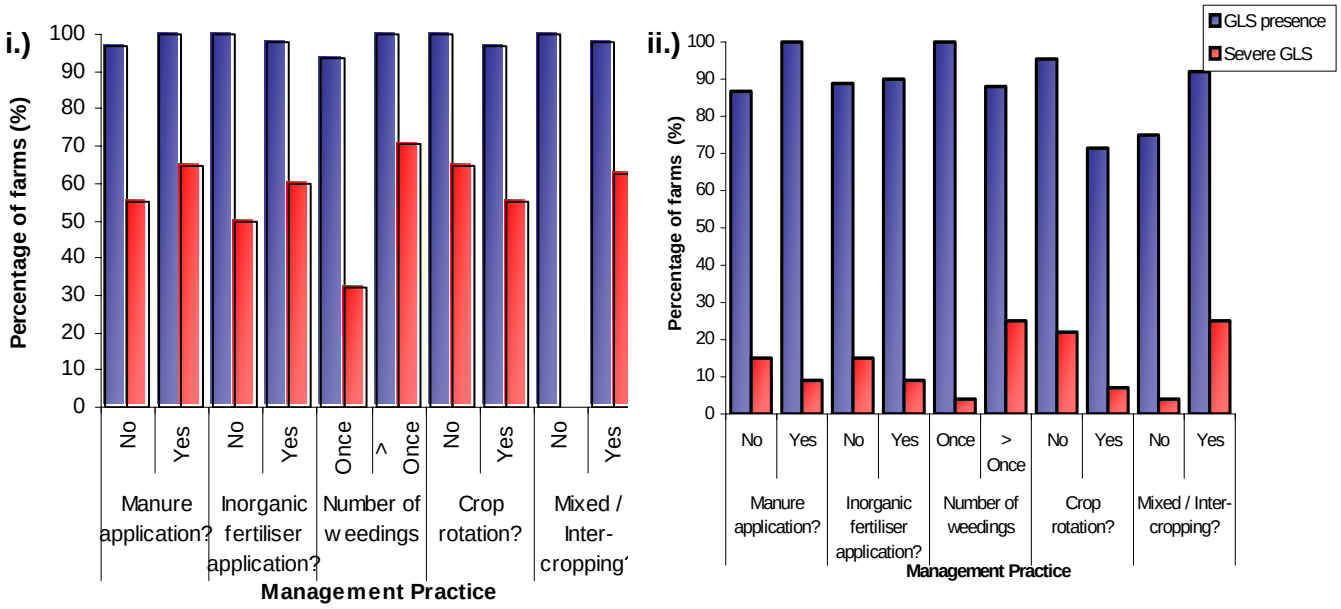


Figure 11: Summary of farms with GLS presence (all levels) and severe levels of GLS described for each level of crop management practice in Zimbabwe (i) and Kenya (ii).

- ***Relationship between post-harvest management practices and incidence/severity of GLS quantified***

Figure 12 shows a summary of relationships between the effects of post-harvest management practices and the incidence/severity GLS. In Zimbabwe, Chi-squared tests did not find any association between severe levels of GLS and maize debris management or soil management

practices. It was not possible to carry out these same tests to compare the incidence of GLS, as expected values for the majority of observations were too low (<5).

However, non-statistical investigation of the data does not highlight any obvious differences. Further modelling using logistic regression in GENSTAT (for yes/no response data) was carried out but no significant crop management practices were found. For the data from Kenya, Chi-squared tests to test for association between post-harvest management practices and GLS presence/severity proved invalid, due to the low number of maize crops observed in Western Kenya.

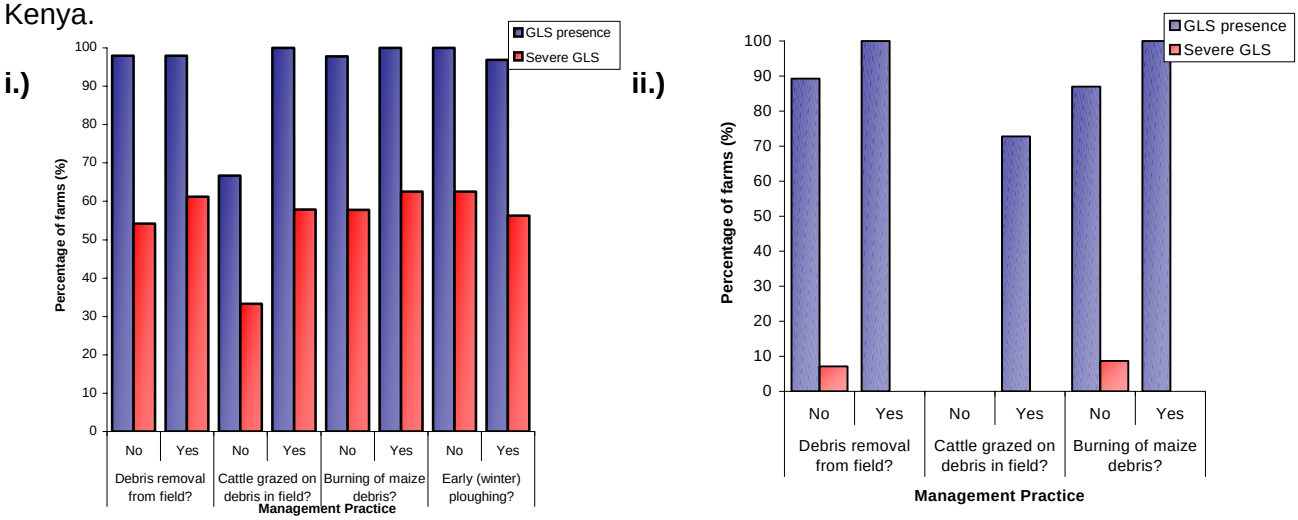


Figure 12: Summary of farms with GLS presence (all levels) and severe levels of GLS described for each level of post-harvest management practice in Zimbabwe (i) and Kenya (ii).

Almost all farmers who fed stover to cattle allowed the cattle onto the field to feed. It is believed that GLS may be spread by cattle and this is the subject of another DFID funded Livestock and Crop Protection Programme project on Integrated Pest Management of Maize and Napier Dairying. In Zimbabwe, few farmers burnt maize debris after harvest (8%) although the majority of farmers carried out post-harvest early (winter) ploughing (65%). It was expected that the burning of maize debris and/or post-harvest early (winter) ploughing would decrease GLS levels because the spores would be either destroyed, by burning, or buried in the soil, by early (winter) ploughing. However, no association was found between GLS levels and post-harvest management practices.

In contrast, in Kenya most farmers left either some, or all, of the maize debris in the field (92%) after harvest. 61% of farmers fed the maize stovers to cattle, the majority allowing the cattle to graze in the field. Burning of maize debris was more common in Kenya, than Zimbabwe. In Kenya, 19% of farmers were burning their maize stovers. This may be because they have received advice from extension workers to burn their debris, but the more likely reason is that burning the debris is more time efficient and also makes ploughing easier. No clear relationship was observed between post-harvest management practices and GLS levels.

- ***Little or no evidence for a relationship between specific insects pests and diseases and GLS***

No clear relationship was observed between specific insect pests and diseases of maize, and GLS in either country, although some evidence of a link between the presence of the insect pest, stem borer and severe levels GLS was observed in Zimbabwe. Researchers also recorded *Phaeosphaeria* leaf spot (PLS) on the majority of farms surveyed in Zimbabwe, and there appears to be some correlation between the two diseases, with severe level GLS occurring alongside severe levels of PLS. However, PLS absence/low-level presence did not imply GLS absence/low level presence, or vice-versa.

Table 1 summarises data for the incidence/severity of GLS on maize crops that suffer from additional, farmer perceived, insect pests and diseases. The numbers of farms in most of the cells are too low to enable a valid chi-squared test for association to be used. Therefore, a relationship

between a particular insect pest or disease and severe GLS would be indicated if the percentage of farms with the specific insect/disease and severe GLS was greater than 59%. This only applied to stem borer - of the sixty-nine farmers who considered stem borer to be a problem, forty-seven (68%) also had severe GLS levels.

Table 1: Summary of incidence/severity GLS on farms where additional insect pests and diseases were noted (number of farms) in Zimbabwe.

		GLS presence on farm			Severe level GLS on farm		
		No	Yes	Total	No	Yes	Total
Insect Pest	Aphids	0	2	2	2	0	2
	Cut worms	0	4	4	2	2	4
	Stem borer	2	67	69	22	47	69
	Termites	0	16	16	8	8	16
Disease	Cob rot	0	6	6	3	3	6
	Streak	1	10	11	6	5	11
	Bacterial Wilt	0	1	1	1	0	1
	Leaf blight	0	1	1	1	0	1

Table 2 summarises the incidence/severity of GLS on maize crops that suffer from additional, farmer perceived, insect pests and diseases in Kenya. The numbers of farms in most of the cells are too low to enable a valid chi-squared test to be used. A general look at the data in the table does not highlight any notable relationships between the insect pests or diseases, and GLS.

Table 2: Summary of incidence/severity GLS on farms where additional insect pests and diseases were noted (number of farms) in Kenya.

		GLS presence on farm			Severe level GLS on farm		
		No	Yes	Total	No	Yes	Total
Insect Pest	Stem Borer	2	14	16	No severe GLS observed on farms where insect pests were mentioned		
	Cut Worms	1	1	1			
	Army Worms	1	0	1			
	Termites	0	1	1			
Disease	Grey Leaf Spot	0	6	6	0	2	2
	Maize Streak Virus	1	8	9	0	0	0
	Smut	1	3	2	0	0	0
	Leaf Blight	0	2	2	0	0	0
	Cob rot	0	3	3	0	2	2
	Stem /Stalk Rot	0	1	1	0	0	0

(N.B. Data on insect pests and diseases were obtained from only 79 of the farmers)

- ***Relationship between GLS incidence and farmer recognition***

On farms where GLS was recognised by farmers it was expected that GLS levels would be high. Table 3 shows that for eleven of the farmers who recognised the disease in Zimbabwe, the level on their farm was severe (i.e. 19% of farmers with severe GLS recognised the disease). However, three farmers with only trace and moderate levels of GLS also recognised the disease. The farmer who had no GLS on his farm, but recognised the disease, had noticed the disease the previous year but had changed to alternative, GLS tolerant, varieties (SC 513, 627, 709) for the 2000/01 growing season.

In Kenya, the number of farms where the maize crop was observed by researchers was small and therefore it is difficult to relate farmer recognition of GLS to actual levels of infection. However, Table 4 shows that for five of the farmers who recognised the disease, the level on their farm was

Table 3: Relationship between farmer recognition of GLS and actual severity on farms in Zimbabwe.

Farmer recognition of GLS	GLS level of severity (number of farms with each level) None	Trace	Low	Moderate	Severe	**Total**
No	1	14	10	12	47	**84**
Yes	1	2	0	1	11	**15**
Total	**2**	**16**	**10**	**13**	**58**	**99**

(N.B. One observation missing as crop unseen)

moderate or severe (i.e. 71%). In addition, two farmers with only trace and low-level GLS also recognised the disease.

Table 4: Relationship between farmer recognition of GLS and actual severity on farms in Kenya.

Farmer recognition of GLS	GLS level of severity (number of farms with each level) None	Trace	Low	Moderate	Severe	**Total**
No	3	4	7	8	0	**22**
Yes	0	1	1	3	2	**7**
Total	**3**	**5**	**8**	**11**	**2**	**29**

(N.B. Maize crop observed on 29 of the 119 farms)

Poor awareness of the disease by small-scale maize farmers and extensionists, together with inadequate knowledge concerning the availability of resistant varieties in Zimbabwe (and lack of resistant varieties in Kenya), are the major constraints to reducing the impact of maize GLS.

- ***Farmer perceptions of GLS and yield losses summarised***

Small-scale farmer awareness of maize GLS is somewhat higher in Zimbabwe than Kenya, due mainly to a greater interaction between the farmers and local agricultural extension officers. The general awareness of GLS, both at the institutional and farmer levels, is related to the presence of large-scale commercial farms in Zimbabwe, which are already tackling the problem of GLS with tolerant maize varieties and chemical controls. The major seed producing companies in Zimbabwe are already promoting GLS-tolerant varieties, however most small-scale farmers continue to be unaware of which varieties are tolerant, and which are susceptible. The largest producers of seed in Zimbabwe (Seed Co., Pannar, Pioneer) market their varieties with a 'score' for GLS tolerance (CABI-ARC, 2002) and provide advice on methods of tackling/preventing yield loss from GLS.

Out of one hundred farmers interviewed in Zimbabwe, fifteen recognised the symptoms of GLS and were aware that it was a disease. A summary of the responses given by these fifteen farmers is provided in Table 5. In addition, eight other farmers had noticed the symptoms of GLS but believed these to be merely the drying out of the leaves or, in one case, related to low fertility. The farmers may believe that the GLS symptoms are drying out of leaves because the disease is coming in late into the crop and therefore is not significantly reducing the yield. A further notable observation is that an additional six farmers, who had previously heard about GLS from agricultural extension workers, seed company field days, and/or from neighbours, and whose farms contained high levels of GLS, did not recognise the disease on their crops.

The majority of farmers, who recognised GLS as a maize disease, were attempting to reduce or control the disease, some taking advice from the local agriculture extension officers (AGRITEX). Ten of the farmers had changed their maize variety to GLS-tolerant varieties since they first started noticing GLS, and two farmers had initiated crop rotation. AGRITEX officers advised one farmer to to plant his maize earlier to avoid onset of the disease. It has been noticed, both in Kenya and

Zimbabwe, that on farms where maize has been planted at different times, the later planted seed shows more severe symptoms of GLS. This can be attributed to a higher reservoir of field inoculum present in the older crop.

Table 5: Summary of farmer perception of GLS in Zimbabwe, severity and yield/financial loss

GLS first noticed in which year?	1998 (2)	1999 (4)	2000 (6)	2001 (2)
Seed variety grown at that time	R 201/215 (1) SC 501 (9)		SC 401 (2) PHB 30A15 (2)	
Year in which GLS was most severe	1999 (1)	2000 (7)	2001 (5)	
Severity in latest crop (2001)	Severe(7)	Moderate(2)	Low/Trace(1)	None(1)
Estimated percentage of yield lost	Ave.= 34.6% (10)		Min.= 0%	Max.= 60%
Estimated financial loss per acre	Ave.=*Z $2,440 (8)		Min.=Z$0	Max.=Z$5,500

(Number of farmers shown in brackets. N.B. some missing observations for certain questions) £1 = Z$78 (at time of data collection)

Recognition of GLS by farmers in Western Kenya was rare. Of the one hundred and nineteen farmers interviewed just 18 said that they recognised the symptoms of GLS and believed it to be present on their farms. Agricultural extension information is limited in Kenya and none of the farmers had heard about GLS from agricultural extension officers. In fact, on one farm, it was discovered that the respondent was himself an extension officer and was unaware of the disease.

Some of the maize varieties available in Kenya are being promoted as GLS-tolerant (e.g. Kenya Seed Company hybrids H614 and H625). However, small-scale farmers were unaware of these varietal traits. Kenya Seed Company has not bred its' current maize varieties for GLS-tolerance, but they are currently evaluating the GLS tolerance of their existing varieties.

A summary of farmer perceptions of GLS in Western Kenya, obtained from the farmers who recognised the disease is given below in Table 6. In addition to the farmers who recognised GLS, four farmers noticed the lesions but thought they were just 'drying out' of the leaves and one farmer believed the lesions were due to low fertility on his farm.

Table 6: Summary of farmer perception of GLS in Kenya, severity and yield/financial loss.

GLS first noticed in which year?	1995 (3)	1997 (4)	1998 (2)	1999 (4)	2000 (3)	
Seed variety grown at that time	H 614 (5) H 625 (2)		H 622 (1) H 627 (4)	Local (4)		
Year in which GLS was most severe	1995 (1)	1996 (1)	1997 (1)	1998 (3)	1999 (5)	2000 (4)
Severity in latest crop (2000 short rains)	Severe(3)	Moderate(2)	Low/Trace(6)	None(4)		
Estimated percentage of yield lost	Ave.= 45.0% (16)		Min.= 0%	Max.= 70%		
Estimated financial loss per acre	Ave.=*Ksh3,900 (14)		Min.=Ksh0	Max.=Ksh10,000		

(Number of farmers shown in brackets. N.B. some missing observations for certain questions) *£1 = Ksh 110 (at time of data collection)

Just four of the farmers who recognised GLS were using methods to control the disease. Each of these farmers had changed maize variety. It is notable that Kenya Seed Company varieties, H614, H625 and H627 are promoted as being GLS-tolerant, however eleven of the farmers who recognised the GLS symptoms stated that these were the varieties that they had planted. This may imply that the varieties are not tolerant, or a more likely explanation is that the farmers were not planting the variety they believed that they were planting. This later explanation is suggested because none of the surveyed farmers was able to show the researchers maize bags with clearly labelled variety names.

Farmer awareness of GLS was greater in Zimbabwe than Kenya. This is likely to be due to the presence of large-scale commercial farmers in Zimbabwe who have been controlling the disease using GLS tolerant varieties and fungicides as well as the seed companies who are involved in research on GLS-tolerant varieties. However, recognition of GLS by farmers in both countries was relatively low, despite the fact that GLS was found on almost all farms in Zimbabwe and the majority of farms in Kenya where maize was observed.

A number of farmers in both countries linked GLS symptoms to the normal drying out of the leaves of the maize while others attributed the symptoms to low soil fertility. This late appearance of the disease, attacks after the grain has already filled, causes very little damage to the maize and therefore no observed yield loss. Farmers in Kenya claimed to have noticed GLS as far back as 1995, whereas in Zimbabwe the farmers interviewed only recognised GLS from 1998.

The number of farmers in both countries who were able to estimate yield loss due to GLS was small and yield loss estimates were not based on empirical evidence. Farmer perception of yield loss was highly variable in both countries (0–70%). On average, farmers estimated a yield loss from GLS of 45% in Kenya and 34.6% in Zimbabwe.

Economic loss calculations show that farmers in Zimbabwe receive a negative gross margin if they do not control for GLS and obtain an approximately zero gross margin if they use fungicide as their GLS-control method, assuming a yield loss due to GLS of 34.6% on susceptible varieties. Fungicide control of GLS is therefore not a viable method for small-scale farmers in Zimbabwe. However, it is clear from the negative gross margin suffered if no control is used that some form of GLS control method should be used.

In Kenya, the use of susceptible varieties with no GLS-control results in a positive gross margin; however, this margin is significantly less than the margin obtained by planting tolerant varieties. Farmers in Kenya using fungicide to control GLS on susceptible varieties obtain a positive gross margin, however, they can increase this margin significantly by using GLS tolerant varieties.

Output 3: Disease epidemiology established through field experimentation supported by the use of molecular markers.

- ***C. zeae-maydis appears to have a narrow host range, effectively limited to maize in certain areas of maize-producing countries.***

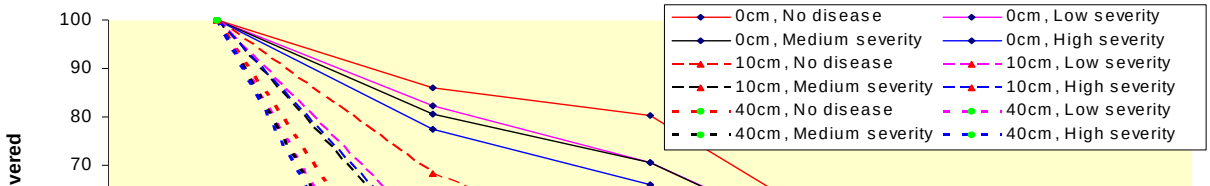
C. zeae-maydis was not isolated from GLS-type lesions on any of the 14 potential alternate hosts tested although molecular analyses using ITS-RFLP confirmed the identity of all of isolates from the potential alternate hosts as *C. sorghii.* Conversely, when isolates of *C. zeae-maydis* were inoculated onto the same 14 potential alternate hosts, they failed to induce GLS-type symptoms.

- **C. zeae-maydis *was not detected in samples of imported maize from the USA***

By matching the fingerprint patterns produced by the DNA from shipments of maize grain to those of pure DNA extracted from cultures of *C. zeae-maydis*, the pathogen was detected in pre-export maize but not in samples from shipments of maize grain imported from the USA, despite the large number of maize grain samples tested. There was considerably more chaff in the pre-export grain, which could explain the ease with which the pathogen was detected.

- ***Evidence for the survivability of GLS in crop debris***

Decay of maize debris was more rapid at depth and with high levels of GLS infection (Fig. 12). The amount of maize debris remaining on the soil surface (0 cm depth) at any one sampling time was significantly higher than the amount recovered from depths of 10 cm and 40 cm, with the latter exhibiting the highest amount of decomposition. This trend was observed across all levels of initial disease severity (Fig. 12). High, initial levels of disease severity levels resulted in high tissue decomposition rates, depicted by large reductions in the amount of debris recovered and *vice versa* (Fig. 12). Significantly more residue was recovered from the disease-free than from the infected leaf material, but leaf portions with low and medium disease levels (all below 10% GLS severity rating) appeared to decompose at similar rates. Debris-decay rates and inoculum load were positively correlated with soil moisture and relative humidity (measured using a data logger: HOBO H8 Pro-Series) – both functions of burial depth.



Burying of maize crop residue clearly accelerates the rate of decomposition compared with stover left on the soil surface. Ploughing to depths of 40cm in order to bury the maize stubble, may be difficult to achieve, however, ploughing depths of 10cm are realistic and will still have some effect on the rates of decomposition of residue.

- ***Clear evidence for the effect of soil fertility on the incidence and severity of GLS***

Incidence and severity of GLS was substantially lower on maize grown in Low N fields than maize grown under 'optimum' conditions (Figure 13) but there was also a significant reduction in yield (Field Trial 1) (Figure 15). As each level of soil fertility i.e. field, has only one replicate, it was not possible to calculate p-values for the effect of fertility (field) alone, however, p-values have been calculated for varietal effects and the interaction between variety and field. In 2001, differences in GLS amongst varieties was significant (Incidence, p=0.045 and 0.049 at silking and grain-filling respectively; Severity, p<0.001 at silking) but no interaction was observed between variety and field (Incidence, p=0.950 and 0.856 at silking and grain-filling respectively; Severity, p=0.798 at silking). Variety X1399AW, which is promoted as a GLS-tolerant variety in Kenya, had the lowest average severity of GLS (Figure 14).

i. **ii.**

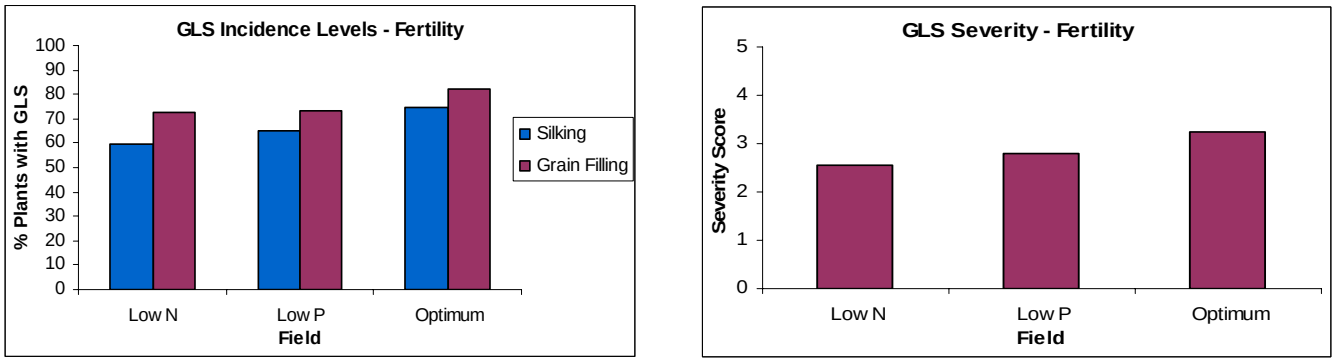
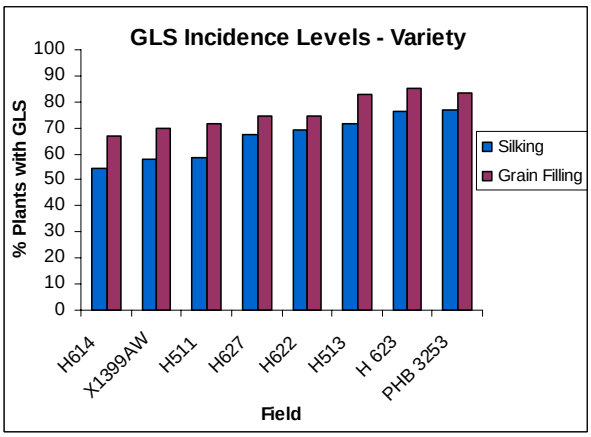


Figure 13: Graphs showing the combined incidence (i) and severity (ii) of GLS in a range of varieties grown at different levels of soil fertility in Kenya, 2001 (Field Trial 1).

Trends in the subsequent yield followed the same pattern, with the 'Optimum' field producing the highest yields and significantly lower yields in the Low N field (Figure 15). There were clear differences between the varieties in terms of yield (p<0.001) (Figure 15) and there was an interaction between variety and field (p=0.003). Incidence and severity of GLS could not be directly correlated with the yield patterns (Incidence, p=0.798 and p=0.668 at silking and grain-filling respectively; Severity, p=0.984 at silking).

i) **ii)**



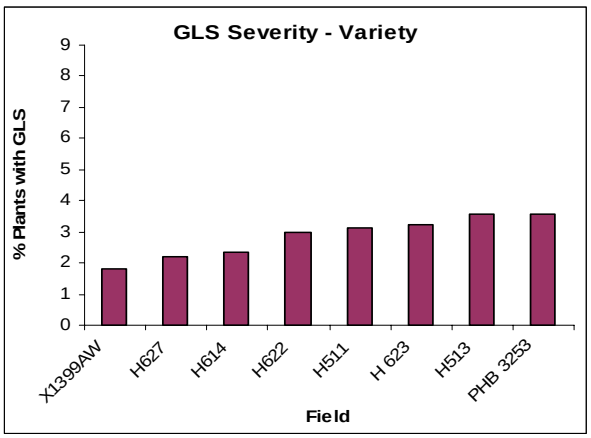
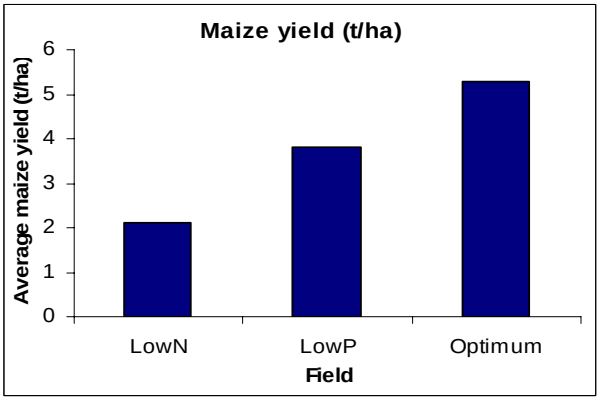


Figure 14: Graphs showing the effect of varieties grown at different levels of soil fertility on the incidence (i) and severity (ii) of GLS at Kakamega, Kenya, 2001 (Field Trial 1)

i)



ii)

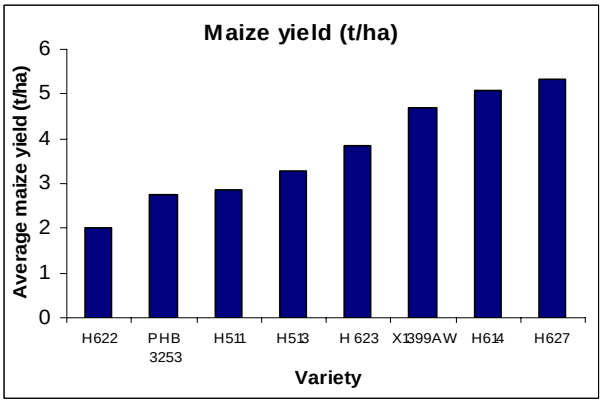


Figure 15: Graphs showing the effect of different levels of soil fertility (i) and variety (ii) on yield at Kakamega, Kenya in 2001 (Field Trial 1)

Lesion progression measurements were taken on two varieties (H614 and X1399AW). Lesion length was generally highest in the 'Optimum' field and lowest in the Low P field. The lesion lengths and rate of progression of the two varieties were not significantly different (p=0.403 and 0.425) (Figure 16).

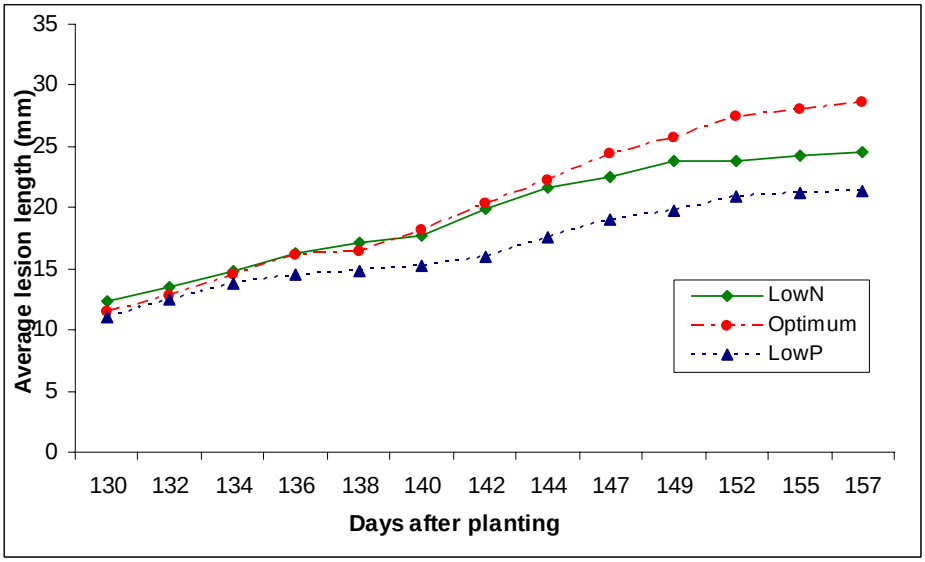


Figure 16: Graph showing the effect of soil fertility on the average dimensions of GLS lesions on two maize varieties (H614 and X1399AW) grown at Kakamega, Kenya in 2001 (Field Trial 1).

Similar data were recorded in 2002 - incidence and severity of GLS was lowest in the Low N field and highest in the 'optimum' nutrient field. Differences in the incidence and severity of GLS among varieties were again significant (Incidence, p<0.001 at grain-filling; Severity, p=0.003 and <0.001 at silking and

grain-filling, respectively) but there was no interaction between variety and field for the severity of GLS (Incidence, p=0.028; Severity, p=0.248 and 0.305 at silking and grain-filling, respectively). As in 2001, the incidence of GLS was lowest in X1399AW but in terms of severity, variety H627 had the lowest severity of GLS in both observations.

The relative yields in the three fields in 2002 were also similar to 2001, and showed clear effects of soil fertility. There were some differences in yield (p=0.059) between varieties but no interaction between variety and level of soil fertility (field) was observed. Again, there was no clear correlation between the yield patterns and the incidence or severity (both observations) of GLS (Incidence, p=0.285 at grain-filling; Severity, p=0.869 and p=0.527 at silking and grain-filling, respectively).

Lesion progression measurements were taken on the same two varieties (H614 and X1399AW). Progression rates and lengths were very similar in all three fields. No significant difference between the two varieties was observed in either length of lesion (p=0.528) or rate of growth (p=0.134).

Similar trends were observed in the Field Trial on soil fertility conducted in Zimbabwe, although the effects of different soil fertility treatments on either the incidence or severity of GLS were not statistically significant in 2002, and in 2003, the levels of GLS were too low to record any differences (Figures 17 and 18).

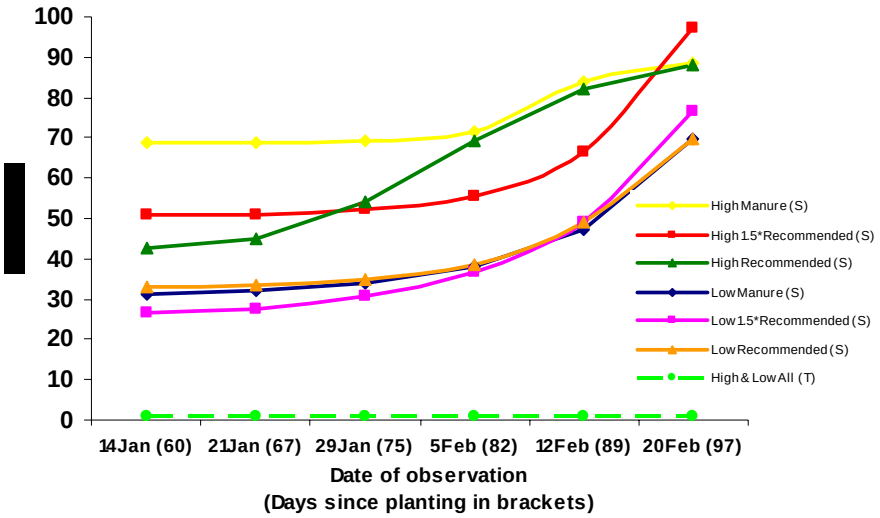
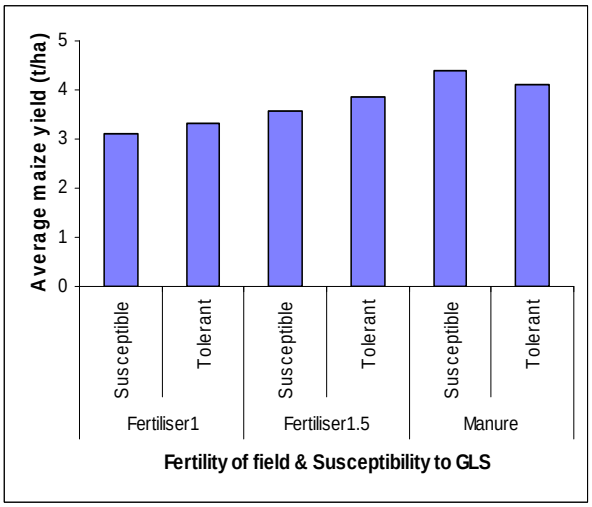


Figure 17 (left): Graph showing the effect of contrasting soil fertility regimes on the incidence of GLS in tolerant (t) and susceptible (s) maize varieties grown under low and high levels of initial inoculum at Henderson Research Station, Zimbabwe in 2001 (Field Trial 2)

Figure 18 (right): Graph showing the effect of contrasting soil fertility regimes on the yield in GLS-tolerant (t) and GLS-susceptible (s) maize varieties grown under low and high levels of initial inoculum at Henderson Research Station, Zimbabwe in 2001 (Field Trial 2)



- ***Evidence for the effect of very early, early and medium maturing maize hybrids on the incidence and severity of GLS***

Similar trends were observed in the Field Trial on the effect of type of maize maturity conducted in Zimbabwe (Field Trial 3), where the effects of different types of maize maturity on either the

incidence or severity of GLS were not statistically significant in 2002, and in 2003, the levels of GLS were too low to record any differences (Figures 19 and 20).

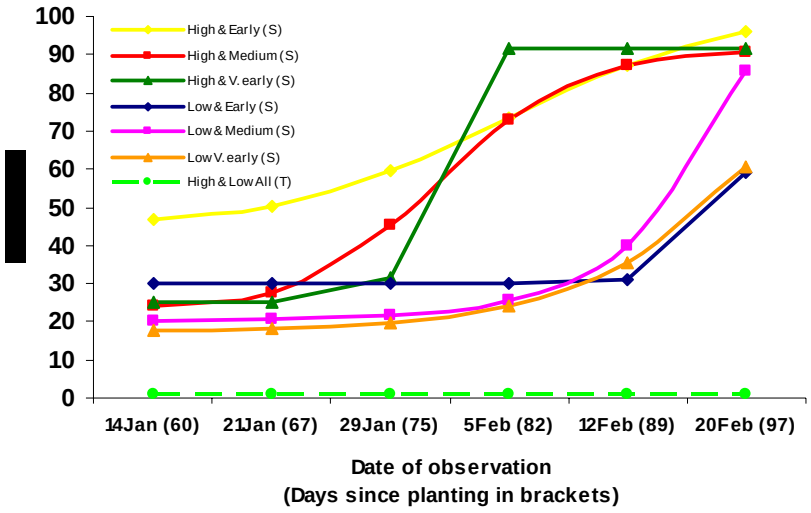
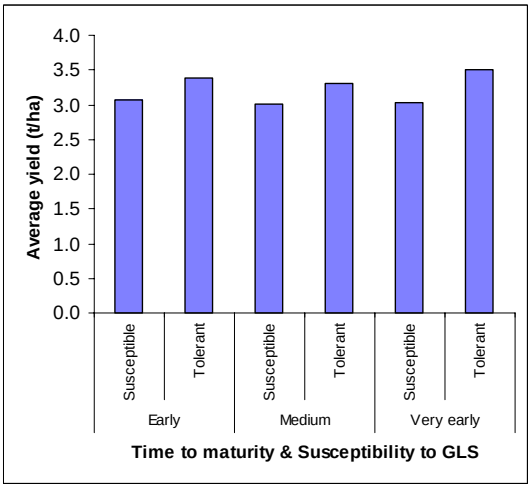


Figure 19 (left): Graph showing the effect of type of maize maturity i.e. very early, early or medium maturing on the incidence of GLS in tolerant (t) and susceptible (s) maize varieties grown under low and high levels of initial inoculum at Henderson Research Station, Zimbabwe in 2001 (Field Trial 3)

Figure 20 (right): Graph showing the effect of type of maize maturity i.e. very early, early or medium maturing on the yield in GLS-tolerant (t) and GLS-susceptible (s) maize varieties grown under low and high levels of initial inoculum at Henderson Research Station, Zimbabwe in 2001 (Field Trial 3)



Output 4: Effective host resistance screening based on pathogen variability.

- ***Effective host resistance screening does not need to incorporate pathogen variability***

Given the high degree of homogeneity (>98%) amongst isolates of *C. zeae-maydis,* the apparent absence of *C. zeae-maydis* Group 1 in Kenya or Zimbabwe (Activity 1.3) and little or no evidence for pathotypic lineages (Activity 1.4) amongst isolates of *c. zeae-maydis* (consistent with the apparent lack of genetic diversity amongst the isolates), at present there is no justification for basing host resistance screening on pathogen variability. Thus, there is little or nothing to be gained by increasing the number of isolates used in resistance screening programmes. This is an encouraging result as it is likely that GLS-resistant varieties on, or entering the market, could potentially be planted across a broad range of maize-growing regions in terms of their performance against GLS.

- ***A rapid protocol for screening for GLS-resistance in the field developed and validated.***

A simplified, rapid protocol for screening for GLS-resistance in the field was developed, and compared with existing protocols currently being used in Kenya and Zimbabwe. The protocol relies on using natural inoculum or 'enhanced' natural inoculum e.g. leaving infested stover from the

previous cropping season on the field into which the new maize crop is to be planted, if the levels of infection are expected to be low. The lack of pathogenic variability amongst isolates of *C. zeae-maydis* (Activity 1.4) precludes the need for producing artificial inoculum consisting of one or more specific isolates. The crop is inspected monthly from the date of planting until tassling, when weekly inspections of the crop are undertaken until crop maturity. For any given variety, there should be at least three replicate plots, consisting of at least 45 sample maize plants (excluding edge rows/plants).

Each sample maize plant is assessed for the severity of GLS using the following, simplified scoring system: 0 = no GLS, 1 = <2% leaf area infected (trace), 2 = 2-10% leaf area infected (low), 3 = 11-50% leaf area infected (moderate), and 4 = >50% leaf area infected (severe).

When compared with existing protocols, the rapid protocol for screening for GLS-resistance in the field developed during the current project was found to be as accurate as other quantitative methods of disease assessment, reduced subjectivity between observers, and in addition was found to be the quickest, simplest and most cost-effective.

The rapid protocol was disseminated to stakeholders i.e. to the national programmes of Kenya and Zimbabwe, CIMMYT and representatives of maize seed companies during the stakeholder workshops.

- ***Available maize germplasm in Kenya and Zimbabwe screened against GLS***

In Zimbabwe the majority of commercially available varieties have been bred for GLS resistance, whereas none of the commercially available varieties in Kenya has been. Results from Field Trial 5 in Kenya reveal significant differences in the severity of GLS among maize varieties in 2001 and 2002, with X1399AW having a lower severity of GLS than all other varieties in 2001($p<0.001$), and varieties X1399AW, H625 and H626 had significantly lower levels of GLS in 2002 ($p=0.026$). H513, KSTP94 and H623 had significantly higher levels of GLS than the other varieties in 2001 whereas in 2002, only variety KSTP94 had a significantly higher severity of GLS.

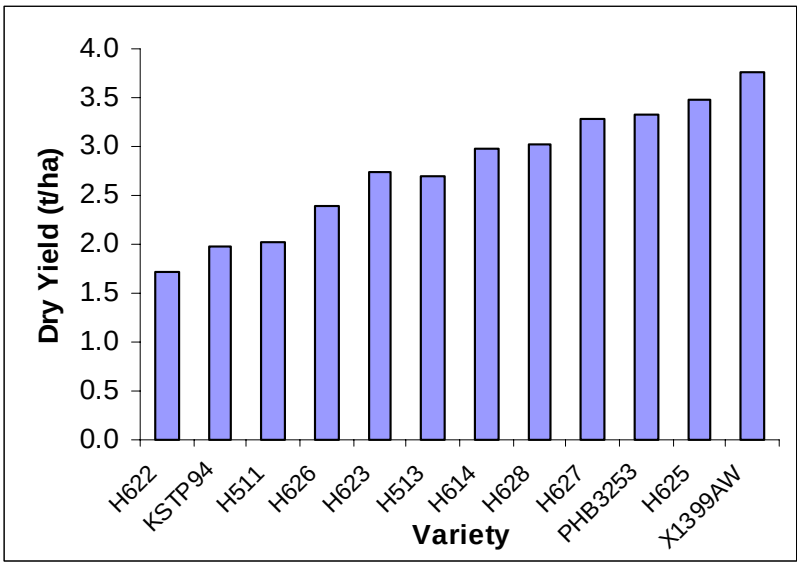


Figure 21 (left): Graph showing the effect of type of maize variety on the yield at Kakamega, Kenya in 2001 (Field Trial 5)

There were also significant differences in subsequent yields ($p<0.001$) in 2001 - varieties H626, 627 and 628 had significantly higher yields than the other varieties (Figure 21), but this effect was not repeated in 2002 ($p=0.322$). There was some evidence of a relationship between yield and GLS such that varieties which had higher severity scores for GLS also produced lower yields, however, after varietal differences had been taken into account this correlation was not significant in either 2001 ($p=0.276$) or 2002 ($p=0.716$).

In Zimbabwe, the severity of GLS at ART Farm in 2002 was extremely low (Figure 22), and there was no significant difference in the effect of variety on the severity of GLS, and in 2003, GLS was too low to score.

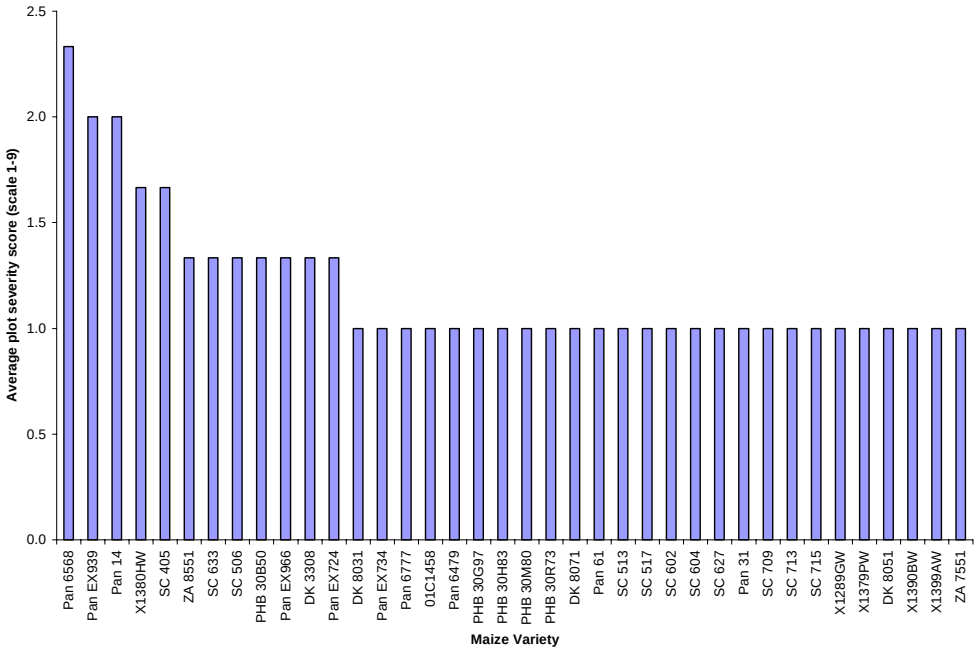


Figure 22: Graph showing the effect of type of maize variety on the Severity of GLS at ART Farm, Zimbabwe in 2001 (Field Trial 6)

- ***Suitable sites for disease resistance screening in Kenya & Zimbabwe identified***

In Kenya, the main 'hot spot' for GLS is in and around Kakamega, whereas in Zimbabwe, the main 'hot spot' for GLS is in and around Harare. Coincidentally, the project collaborators, KARI, have a large field station at Kakamega, which provides an ideal site for screening for resistance to GLS. Similarly, in Zimbabwe, PPRI's Henderson Field Station and ART Farm are both located in the GLS 'hot spot' making those ideal locations for screening maize for resistance to GLS.

Maize germplasm showing resistance/tolerance to GLS in one location, especially a GLS-hotspot could potentially be used against GLS in a wide range of environments, provided that the maize hybrid/variety is adapted to these conditions. Where a particular maize variety is not adapted to the conditions prevalent in the GLS-hotspots, they could be screened for resistance to GLS at alternative sites. However, as the development of GLS is highly dependent on environmental effects, if such sites are not important for GLS, then it is questionable whether or not such data would be useful in the context of a disease resistance screening trial!

- ***GLS screening technologies incorporated into national maize breeding/selection programmes***

In both Kenya and Zimbabwe, the National Programmes had already developed and incorporated into their maize breeding/selection programme, technologies for resistance to maize GLS by the time the current project was initiated. CIMMYT, which has regional offices in Nairobi, Kenya and Harare, Zimbabwe also includes screening for maize GLS in its characterisation of maize germplasm grown in Eastern and Southern Africa. Most of CIMMYT's breeding/selection programmes are carried out in close collaboration with the National Programmes, particularly in Kenya. Similarly, the commercial maize seed companies, especially those in Zimbabwe, have been screening their maize germplasm for GLS since 1997, and more recently, screening for GLS has being carried out by commercial seed companies in Kenya. During the course of this project, maize breeders from the public and private sector in Kenya and Zimbabwe were visited to discuss current screening technologies for GLS, and at the 'end-of-project' workshop, there was excellent participation from maize breeders working for the National Programmes of Kenya and Zimbabwe, together with maize breeders from CIMMYT and the four major maize seed companies in Zimbabwe i.e. SeedCo, Pannar, Pioneer and Monsanto. Information concerning the 'rapid' maize GLS screening technology, developed by this project, has been promoted to maize breeders (National Programme, CIMMYT and Commercial Seed Companies) in three African countries, South Africa, Kenya and Zimbabwe.

Output 5: Improved cultural practices for disease control validated on farm.

- ***Awareness of GLS by small-scale maize farmers and extensionists in Kenya and Zimbabwe raised.***

The project has had a significant impact on the awareness of GLS amongst small-scale maize farmers and extensionists in Kenya and Zimbabwe, through stakeholder workshops, direct contact with project researchers during the biological and socio-economic surveys, and training exercises ('awareness-raising') with >50 extension staff and >500 small-scale maize farmers. A poster on 'Raising awareness of maize GLS' was produced in Swahili, Shona and English versions (see Appendix 5) and disseminated to >5000 small-scale farmers and extensionists. The poster generated a considerable amount of interest and there have been numerous requests for more copies. Of particular note were the requests from representatives of the commercial maize seed companies in Kenya and Zimbabwe. It would certainly be good if additional funding could be made available to allow more posters to be printed and disseminated. In addition, training videos on 'recognising GLS' and farmers perceptions of the disease have been produced in Swahili and Shona, and used in raising awareness of the disease.

- ***Preliminary recommendations for an IPM Strategy for GLS developed and promoted to small-scale maize farmers and extensionists in Kenya and Zimbabwe***

Using a combination of information from existing literature, data from the biological and socio-economic surveys together with the results of experimental work carried out during the current project, a preliminary IPM strategy for maize GLS was developed. The strategy was designed specifically for the target beneficiaries i.e. small-scale maize farmers and extentionists (Governmental and non-Governmental), and was based on a 'basket of options' including the use of improved cultural practices i.e. crop rotation, management of crop debris and soil fertility, together with the use of resistant varieties. The IPM strategy for GLS was promoted through stakeholder workshops, training exercises (GLS-management options) with >50 extension staff and >500 small-scale maize farmers and through the production of a leaflet on 'Options for managing maize grey leaf' in Swahili, Shona and English versions (see Appendix 6) which has been disseminated to >1500 small-scale maize farmers and extensionists in Kenya and Zimbabwe.

- ***IPM strategy for the management of maize GLS by small-scale farmers validated through farmer-participatory training***

Plate 10: Farmers voting on the acceptability of the IPM Strategy

The acceptability and effectiveness of the IPM strategy for the management GLS by small-scale maize farmers, including an 'awareness-raising' activity, use of improved cultural practices together with the use of resistant varieties was validated during training exercises, farmer-participatory training sessions and on-going farmer field schools in two contrasting districts of Kenya. The 'basket of options' for the IPM strategy includes cultural practices i.e. removal/burial of infested maize debris, soil fertility, fungicide application (Zimbabwe) as well as the use of resistant varieties. As there were only two complete maize-growing seasons during the course of the project, it was not possible to include farmer-participatory experimentation following the development of the IPM strategy. Nevertheless, the strategy has been successfully incorporated into on-going farmer participatory activities through existing linkages with other projects e.g. IFAD-funded Farmer Field Schools in Maize-based cropping systems and FAO-co-ordinated Farmer Field Schools in Central Kenya.


Plates 11 and 12: Small-scale maize farmers in Western Kenya validating the IPM strategy for maize GLS

Contribution of Outputs to Developmental Impact

All five of the project outputs have been achieved! As a result, we now have a much clearer understanding of the variability (or lack of) of the pathogen population in Africa, the epidemiology of the disease and management strategies which are appropriate for the target beneficiaries' i.e. small-scale maize farmers. Of particular importance is the development of resistant maize varieties which are very early/early maturing and therefore preferred by many small-scale farmers who rely on rain-fed irrigation. Moreover, many of the new, resistant varieties no longer carry a price premium above the traditional varieties. By incorporating the use of resistant varieties into an integrated strategy for the management of maize GLS, which includes 'awareness raising' as well as utilising beneficial cultural practices, the outputs have contributed to the overall project goal by developing and promoting an IPM Strategy for Maize GLS by small-scale farmers in Eastern and Southern Africa. In summary, the IPM Strategy for Maize GLS will 'improve and enhance sustainability of yields by a cost-effective reduction in losses due to pests'.

List of Publications:

- CAB International – Africa Regional Centre (2002) Socio-economic survey of Maize Grey Leaf Spot on small-scale maize farms in Kenya and Zimbabwe: Farmer Perceptions and Economic Analysis. 46pp.
- Kinyua, Z.M. (2003) *Genetic structure and virulence characterisation of Cercospora populations causing maize grey leaf spot in Kenya.* PhD Thesis, University of London.

Internal Reports:

Progress Reports (2000-2001)

- Simons, S.A. *Quarterly Report (Q1) April-June 2000*
- Simons, S.A. *Quarterly Report (Q2) July-September 2000*
- Simons, S.A. *Quarterly Report (Q3) October-December 2000*
- Simons, S.A. *Annual Report: 1 April 2000 – 31 March 2001*

Progress Reports (2001-2002)

- Simons, S.A. *First Progress Report (PPR-1) April-September 2001*
- Simons, S.A. *Second Progress Report (PPR-2) October-December 2001*
- Simons, S.A. *Annual Report: 1 April 2001 – 31 March 2002*

Progress Reports (2002-2003)

- Simons, S.A. *First Progress Report (PPR-1) April-September 2002*
- Simons, S.A. *Second Progress Report (PPR-2) October-December 2002*
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- Simons, S.A., Oduor, G.I. & Ajanga, S.* (2000) *Back-to-Office Report on a nationwide biological survey of Maize Grey Leaf Spot in Kenya, 1-30 September 2000*. CAB International – Africa Regional Centre (CABI-ARC). 2pp.
- Sithole, S. & Mguni, C. (2000) *Back-to-Office Report on a visit to Kenya to participate in a stakeholder workshop on Maize Grey Leaf Spot at CAB International – Africa Regional Centre, Nairobi, 11-14 October 2000*. Plant Protection Research Institute (PPRI). 2pp.

- Oruko, L., Poole, J. & Ajanga, S.* (2001) *Back-to-Office Report on a nationwide socio-economic survey of Maize Grey Leaf Spot in Kenya, 15 January-12 February 2001*. CAB International – Africa Regional Centre (CABI-ARC) and * Kenya Agriculture Research Institute (KARI). 2pp.
- Simons, S.A. (2001) *Back-to-Office Report on a visit to Zimbabwe & South Africa to undertake a nationwide biological survey of Maize Grey Leaf Spot in Zimbabwe and visit on-going field trials on Maize Grey Leaf Spot at Cedara Research Station, KwaZulu Natal Department of Agriculture, South Africa, 10-27 March 2003*. CAB International – Africa Regional Centre (CABI-ARC). 3pp.

Back to Office Reports (2001-2002)

- Kinyua, Z.M. (2001). *Back-to-Office Report on a visit to CAB International - UK Centre for training in the use of molecular techniques to characterise Kenyan isolates of* Cercospora zeae-maydis, *2001*. Kenya Agricultural Research Institute (KARI). 2pp.
- Mtisi, E. (2001). *Back-to-Office Report on a visit to CAB International - UK Centre for training in the use of molecular techniques to characterise Zimbabwean isolates of* Cercospora zeae-maydis, *2001*. Kenya Agricultural Research Institute (KARI). 2pp.
- Njuki, J. (2001) *Back-to-Office Report on a visit to Zimbabwe to complete the socio-economic survey of Maize Grey Leaf Spot in Zimbabwe, 3-7 September 2001*. CAB International – Africa Regional Centre (CABI-ARC). 2pp.
- Poole, J. (2001) *Back-to-Office Report on a visit to Zimbabwe to participate in a nationwide socio-economic survey of Maize Grey Leaf Spot in Zimbabwe, 2-13 April 2001*. CAB International – Africa Regional Centre (CABI-ARC). 2pp.
- Simons, S.A. & Ajanga, S. *Back-to-Office Report on a visit to Zimbabwe to plan field trials for the 2001-2002 season, 3-7 September 2001*. CAB International – Africa Regional Centre (CABI-ARC) and *Kenya Agriculture Research Institute (KARI). 3pp.
- Simons, S.A. (2001) *Back-to-Office Report on a visit to Zimbabwe to identify sites and initiate field trials for the 2001-2002 season, 23-28 September 2001*. CAB International – Africa Regional Centre (CABI-ARC). 2pp.

Back to Office Reports (2002-2003)

- Karanja, D. & Chandi, E. (2002) *Back-to-Office Report on a Follow-up Biological Survey on Maize Grey Leaf Spot in Western Kenya*, *12–23 June 2002*. CAB International – Africa Regional Centre (CABI-ARC). 2pp.
- Kinyua, Z.M., Ajanga, S. & Kalya, B.C. (2002) *Back-to-Office Report on the Second Round of a Biological Survey on Grey Leaf Spot Disease*, *12–23 June 2002*. Kenya Agricultural Research Institute (KARI). 3pp.
- Karanja, D. & Chandi, E. (2002) *Back-to-Office Report on a Follow-up Biological Survey on Maize Grey Leaf Spot in Central Eastern & Coastal Regions of Kenya*, *24 July-2 August 2002*. CAB International – Africa Regional Centre (CABI-ARC). 2pp.
- Kinyua, Z.M. (2002). *Back-to-Office Report on a visit to CAB International - UK Centre to undertake activities on the characterisation of isolates of* Cercospora zeae-maydis, *30 September 2002 – 6 December 2002*. Kenya Agricultural Research Institute (KARI). 3pp.

- Kibata, G., Ochieng, J., Ajanga, S. & Kinyua, Z.M. (2003) *Back-to-Office Report on a visit to Zimbabwe to participate in a Stakeholder Workshop entitled, 'Maize Grey Leaf Spot: A problem solved?' 3-10 March 2003*. Kenya Agricultural Research Institute (KARI). 3pp.
- Simons, S.A. (2003) *Back-to-Office Report on a visit to Zimbabwe to monitor on-going field trials at Henderson Research Station and initiate preparations for the end-of-project workshop, 5-8 February 2003*. CAB International – Africa Regional Centre (CABI-ARC). 2pp.
- Simons, S.A. (2003) *Back-to-Office Report on a visit to Zimbabwe to sample field trials and analyse data*, *19-21 February 2003*. CAB International – Africa Regional Centre (CABI-ARC). 2pp.
- Simons, S.A., Njuki, J. & Poole, J. (2003) *Back-to-Office Report on a visit to Zimbabwe to plan and host a Stakeholder Workshop entitled, 'Maize Grey Leaf Spot: A problem solved?' 3-10 March 2003*. CAB International – Africa Regional Centre (CABI-ARC). 2pp.

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- Ajanga, S. (2001) Report on field trials on Maize Grey Leaf Spot at Kenya Agricultural Research Institute (KARI) Kakamega Centre in 2001. 12pp.
- Mtisi, E. (2001) *Genetic structure and virulence characterisation of Cercospora populations causing maize grey leaf spot in Zimbabwe*. A Scientific Report. 25pp.
- Ajanga, S. (2002) Report on field trials on Maize Grey Leaf Spot at Kenya Agricultural Research Institute (KARI) Kakamega Centre in 2002. 10pp.

Other Dissemination of Results:

External Reports

- Kinyua, Z.M. (2001) *Genetic structure and virulence characterisation of Cercospora populations causing maize grey leaf spot in Kenya*. A First Year Report for MPhil/PhD Studies at Royal Holloway, University of London, 26 June 2001. 39pp.
- Kinyua, Z.M. (2002) *Genetic structure and virulence characterisation of Cercospora populations causing maize grey leaf spot in Kenya*. A Second Year Report for PhD Studies at Royal Holloway, University of London, 21 October 2002. 38pp.

Farmer Field Day

- Promoting strategies for the management maize grey leaf spot by small-scale maize farmers in Western Kenya, March 2003. CAB International – Africa Regional Centre (CABI-ARC), Kenya Agricultural Research Institute (KARI) and the Global IPM Facility.

Leaflets

- Options for managing maize grey leaf spot: a leaflet for small-scale farmers in Kenya (Swahili version). CAB International – Africa Regional Centre (CABI-ARC) and Kenya Agricultural Research Institute (KARI).
- Options for managing maize grey leaf spot: a leaflet for small-scale farmers in Zimbabwe (Shona version). CAB International – Africa Regional Centre (CABI-ARC) and Plant Protection Research Institute (PPRI).
- Options for managing maize grey leaf spot: a leaflet for small-scale farmers in Eastern and Southern Africa (English version). CAB International – Africa Regional Centre (CABI-ARC).

Open Days

- Kenya Agricultural Research Institute (KARI) – Kakamega Centre, September 2001.
- Kenya Agricultural Research Institute (KARI) – Kakamega Centre, September 2002.

Oral Presentation

- Simons, S.A. (2001) Lecture on Maize Grey Leaf Spot given to University of Natal, South Africa, 26 March 2001.

Posters

- Raising awareness of Maize grey leaf spot: a poster for small-scale farmers in Kenya (Swahili version). CAB International – Africa Regional Centre (CABI-ARC) and Kenya Agricultural Research Institute (KARI).
- Raising awareness of Maize grey leaf spot: a poster for small-scale farmers in Zimbabwe (Shona version). CAB International – Africa Regional Centre (CABI-ARC) and Plant Protection Research Institute (PPRI).
- Raising awareness of Maize grey leaf spot: a poster for small-scale farmers in Eastern and Southern Africa (English). CAB International – Africa Regional Centre (CABI-ARC).

Proceedings

- Simons, S.A. (2000) *Proceedings of a stakeholders workshop on Maize Grey Leaf Spot. 12-13 October 2000*. CAB International – Africa Regional Centre. 16pp.
- Simons, S.A. (2003) *Proceedings of a stakeholder's workshop entitled, 'Maize Grey Leaf Spot: A problem solved?' 6-7 March 2000*. CAB International – Africa Regional Centre. 25pp.

Videos

- A video recording of Farmer Perceptions of Maize Grey Leaf Spot in Western Kenya, February 2003.
- A video recording of Farmers Perceptions of Maize Grey Leaf Spot in Central & Eastern Zimbabwe, March 2003.

Workshops

- Stakeholders Workshop on Maize Grey Leaf Spot held at CAB International – Africa Regional Centre, Nairobi, Kenya, 12 & 13 October 2000.
- Stakeholders Workshop on Maize Grey Leaf Spot: A problem solved? Held at Mandel Training Centre, Harare, Zimbabwe, 6 & 7 March 2003.

Given the 'basket of options' available for managing the disease, additional strategic research at this stage is not warranted, and has not been planned. However, there are two key issues highlighted in the socio-economic surveys, which urgently need to be addressed as a follow-up to the current project. Firstly, awareness of the disease amongst small-scale maize farmers and extensionists continues to be surprisingly low. It was this finding which prompted the production of posters in Swahili, Shona and English, to raise awareness of the disease. This was certainly an excellent start in addressing the problem, but considerably more now needs to be done. Secondly, despite the widespread availability of maize varieties which are resistant to GLS in Zimbabwe, awareness of the existence of resistant varieties and other management strategies continues to be poor, and there is also a marked reluctance by some small-scale farmers to switch from their 'traditional' varieties to the newer, resistant maize varieties.

It is the opinion of the author that both of the aforementioned constraints could be effectively addressed by a relatively short-term (e.g. 1-year), 'promotional project' involving training of extensionists (Governmental and non-Governmental), production of additional farmer-friendly dissemination materials e.g. production of posters showing potential management strategies for maize GLS, and by visiting and providing input into existing farmer-field schools and other farmer groups in key maize-growing areas in Kenya and Zimbabwe. It is, therefore, recommended that the Crop Protection Programme considers funding a follow-up, 'promotional project' to ensure that the positive and beneficial outcomes from the current project on maize GLS have greater impact on a larger number of target beneficiaries.

Recommendations and Conclusions

In the 1995 maize-growing season, when GLS was first reported on maize in Kenya and Zimbabwe, farmers experienced significant yield losses, and methods for controlling the disease were not readily available. Most of the maize varieties being cultivated at that time were highly susceptible to GLS, and little was known or understood about the epidemiology of the disease in Africa. As a consequence, management strategies which were appropriate for small-scale maize farmers in Africa, had not been developed. Furthermore, evidence from other maize-producing countries around the world indicated that under conducive environmental conditions, GLS could result in total yield loss, and thus posed a serious threat to food security in Africa. It was in response to this situation that in 1999, DFID's Crop Protection Programme put out a call for proposals to investigate potential management strategies for GLS, which resulted in the approval and subsequent funding of the current project. The project was implemented from 2000-2003 by CAB *International*, in close collaboration with two partner institutes i.e. Kenya Agricultural Research Institute (KARI), Kenya and the Plant Protection Research Institute (PPRI) of the Department of Research and Scientific Services, Zimbabwe.

The overall 'purpose' of the project i.e. 'to provide effective management of GLS based on sound epidemiological principles,' Was achieved through the successful delivery of the five key outputs: 1. Variability of the pathogen population determined using representative isolates from both Kenya and Zimbabwe; 2. Cultural practices affecting GLS incidence and severity identified; 3. Disease epidemiology established through field experimentation and supported by the use of molecular markers; 4. Effective host resistance screening based on pathogen variability; and 5. Improved cultural practices for disease control validated on farm.

Knowledge generated during the current project, together with existing literature, has been used to design, develop and promote an integrated pest management strategy for GLS, appropriate for small-scale maize farmers in Africa. The strategy is based on a combination of, 'raising awareness' of disease coupled with a 'basket of options' for the effective management of the disease. In summary:

- Of paramount importance in managing GLS, is the ability to recognise the disease, and to be aware of the potential yield losses caused by GLS. This requires an effective **awareness-raising campaign** (Appendix 5)

- Where the disease does occur, there is a **basket of options** appropriate for small-holder maize farmers which can be incorporated into an effective integrated strategy for managing the disease – an IPM strategy for GLS (Appendix 6)!

  Options for managing maize GLS

  - If you have had a severely affected crop, avoid planting maize back onto the same piece of land for at least one season.

  If this is not possible, combine the following measures:

  - Any maize debris left over from the infected crop should be buried deeply, well before you plant the next maize crop; burying the debris soon after harvest is preferable.
  - Avoid use of excessive nitrogen (more than recommended rates) as this encourages disease severity.
  - Plant resistant varieties/hybrids. There are now many GLS-resistant maize varieties which are now commercially available and cost no more than the maize hybrids grown by the majority of small-scale maize farmers.

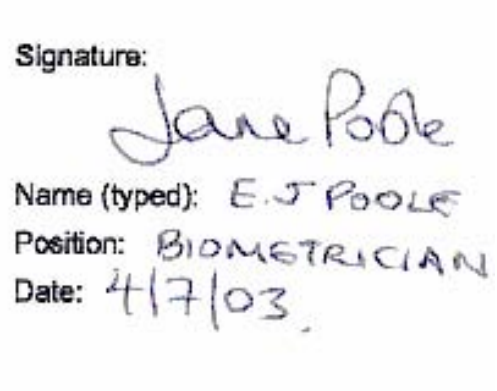
It is recommended that the IPM Strategy for managing maize GLS, developed during the current project, is now promoted to target beneficiaries' i.e. small-scale maize farmers, on a much larger scale and over a broader geographic area i.e. throughout East, Central and Southern Africa. This

can best be achieved through the distribution of the posters and leaflets produced by the current project, targeted training of extension officers and close interaction with on-going projects and community-based activities involved in the uptake and adoption of new knowledge and/or technologies for improving the productivity of maize e.g. Farmer Field Schools.

In conclusion, although it may be premature to consider GLS as a 'problem solved', the availability of affordable, GLS-resistant maize varieties, together with appropriate management strategies means that GLS is a problem which can be effectively managed through a good IPM strategy!

Biometricians Signature

I confirm that the biometric issues have been adequately addressed in the Final Technical Report:



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Appendices

Appendix 1: Description of agro-ecological zones in Kenya

Source: Farm Management Handbook of Kenya

Belt	Zone	Elevation	Temperature	Precipitation (% of potential evaporation)	Maize production potential
Lower Highland	Humid	1,800-2,2400m	Moderately cool, annual mean	> 80	Fair [3]
	Subhumid			65-80	Fair
	Semi-humid			50-65	Fair - marginal
Upper Midland	Humid	1,300-1,900m	Temperate, annual mean 18-21°C	> 80	Good – Fair
	Subhumid			65-80	Good [2]
	Semi-humid			50-65	Fair [1]
	Transitional			40-50	Fair [1]
Lower Midland	Humid	800-1,500m	Warm, annual mean 21-24°C, mean min, >14°C	> 80	Fair
	Subhumid			65-80	Good
	Semi-humid			50-65	Fair [1]
	Transitional			40-50	Fair – Poor [4]

[1] depending on soils [2] Good = ave. yield >60% of optimum on suitable soils,
[3] Fair = ave. yield 40-60% of optimum on suitable soils [4] Poor = average yield 20-40% of optimum on suitable soils.

Appendix 2: Description of agro-ecological (Natural) regions in Zimbabwe.

I Specialized and Diversified Farming Region:
Rainfall is high (>1000mm per annum at altitudes <1,700m, >900mm per annum at altitudes >1,700m), normally with precipitation in all months. Temperatures comparatively low and rainfall consequently effective in enabling afforestation, fruit and intensive livestock production to be practiced. In forest-free areas plantation crops such as tea, coffee and macadamia nuts can be grown.

II Intensive Farming Region:
Rainfall confined to summer and moderately high (750 – 1000mm). Two sub-regions have been defined.
IIA received average of at least 18 rainy pentads per season and normally reliable conditions, rarely experiencing severe dry spells in summer. Region is suitable for intensive systems of farming based on crops and/or livestock production.
IIB receives an average of 16-18 rainy pentads per season and is subject to rather more severe dry spells during the rainy season or to the occurrence of relatively short rainy seasons. Crop yield in certain years will be affected, but not sufficiently frequently to change the overall utilization from intensive systems of farming.

III Semi-Intensive Farming Region:
Rainfall is moderate in total amount (650 – 800mm), but, because much of it is accounted for by infrequent heavy fall and temperatures are generally high, its effectiveness is reduced. Region will receive average of 14-16 rainy pentads per season. Region also subject to fairly severe mid-season dry spells and is therefore marginal for maize, tobacco and cotton production.

IV Semi-Extensive Farming Region:
Fairly low total rainfall (450 – 650mm) and is subject to periodic season droughts and severe dry spells during the rainy season. Rainfall is too low and uncertain for cash cropping except in certain very favourable localities, where limited drought-resistant crops can afford a sideline.

V Extensive Farming Region:
Rainfall in this region is too low and erratic for the reliable production of even drought-resistant fodder and grain crops. Included in this region are areas <900m altitude, where the mean rainfall is <650mm, in the Zambezi valley and <600mm, in the Sabi-Limpopo valleys.

Source: Department of Agricultural, Technical and Extension Services (AGRITEX)

Appendix 3: Questionnaire for a Socio-economic Survey for Maize Grey Leaf Spot in Kenya

Data collectors S. Ajanga (KARI-Kakamega), L.O. Oruko and J. Poole (CABI-ARC)

Date:_____

1.GPS coordinates: _____ 2.District :_____ 3. Village/Location:_____

4.Name of farmer: _____ M / F 5.Total farm size (acres):_____ 6. Maize crop area (acres): ____

7.Maize varieties grown in the last year:

Season	Variety/Varieties	Seed source(s)
L.Rains		
S.Rains		

8. Maize management practices:

Ploughing	Tractor / Hand / Draught / None
Weeding	Number: Method:
Organic Manure	Yes / No
Fertilizer	Yes / No When: Planting Top dressing
Chemicals	Yes / No Herbicide / Insecticide / Fungicide
Rotation	(last 2 years) 1. 2.
Relay cropping	Yes / No If Yes – what?
Intercropping	Yes / No If Yes – what?
Maize debris removal method	Removed / Left on field Plough / Burn Grazed by cattle: Yes / Winter ploughed: Yes / No

9.Do you recognize GLS as a disease affecting your maize crop? Yes / No (show pictures or sample for identification)

If No then interview finishes here. If Yes, then continue.

10. Year you first noticed GLS on crop? 19__ ___ SR / LR 11.Variety grown at that time:_____

12. In which year was GLS most severe? 19____ SR / LR Severity: Low / Medium / High

13. Estimate the severity of GLS in your last crop: None / Low / Medium / High

14. What was your maize yield, per acre (in the year given in Qu 12.)? _____ (Bags)

15. Estimate yield lost, per acre due to GLS damage (in the year provided in Qu 12.)? _____ (Bags)

16. What is the price you receive for 1bag (90kg) of maize? Ksh _____

17. Have you used any of the following to control the GLS?

Management Practice	**X**	If yes, then describe…
Change of maize variety		Varieties:
1 year crop rotations		
Burning of maize debris		
Turning of maize residues into the soil		
Use of fungicides (& cost)		Name:
Other		

18. What are the other main pests and diseases that affect your maize crop? (To be answered by all farmers)

Pests	Rank

Diseases	Rank
~~Grey Leaf Spot~~	

Appendix 4: Questionnaire for a Socio-economic Survey for Maize Grey Leaf Spot in Zimbabwe

Data collectors: M.Chimbira (AGRITEX), M.Madondo & E.Mtisi (PPRI), J. Poole (CABI-ARC)

Date:_____

1.GPS coordinates: _____ 2.District :_____

3.Communal/Village:_____

4.Name of farmer: _____ M / F 5.Total farm size (acres):_____ 6. Maize crop area (acres): ____

7.Maize varieties grown in the last year:

Variety/Varieties	Seed source(s)

8. Maize management practices:

Ploughing	Tractor / Hand / Draught / None
Weeding	Number: Method:
Organic Manure	Yes / No
Fertilizer	Yes / No When: Planting Top dressing
Chemicals	Yes / No Herbicide / Insecticide / Fungicide
Rotation	(last 2 years) 1. 2.
Relay cropping	Yes / No If Yes – what?
Intercropping	Yes / No If Yes – what?
Maize debris removal method	Removed / Left on field Plough / Burn Grazed by cattle: Yes / Winter ploughed: Yes / No

9.Do you recognize GLS as a disease affecting your maize crop? Yes / No (show pictures or sample for identification)

If No then interview finishes here. If Yes, then continue.

---

10. Year you first noticed GLS on crop? _____ Growth stage _____ 11.Variety grown at that time:_____

12. In which year was GLS most severe? _____ Severity: Low / Medium / High

13. Estimate the severity of GLS in your last crop: None / Low / Medium / High

14. What was your maize yield, per acre (in the year given in Qu 12.)? _____ (Bags)

15. Estimate yield lost, per acre due to GLS damage (in the year provided in Qu 12.)? _____ (Bags)

16. What is the price you receive for 1bag (50kg) of maize? Z$ _____

17. Have you used any of the following to control the GLS?

Management Practice	**X**	If yes, then describe…
Change of maize variety		Varieties:
1 year crop rotations		
Burning of maize debris		
Turning of maize residues into the soil		
Use of fungicides (& cost)		Name:
Other		

18. What are the other main pests and diseases that affect your maize crop? (To be answered by all farmers)

Pests	Rank

Diseases	Rank

HAVE YOU SEEN THIS DISEASE?

It is

Maize Grey Leaf Spot (GLS)

INFECTED Maize

HEALTHY Maize

Symptoms:

Young GLS lesions are small tan spots or short, straight lines.

Mature GLS lesions are tan or grey in colour and are clearly rectangular.

Produce cobs like this...

For more information contact:
Dept. of AREX, Plant Protection Research Institute, 5th Str Extension, P.O. Box CY 500, Causeway, Harare.
Tel. +263 4 704531/700339
OR
CABI-ARC, P.O. Box 633-00621 Village Market, Nairobi, KENYA.
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