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Interactive effects of host, pathogen and mineral nutrition on grey leaf spot epidemics in Uganda

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Abstract

Grey leaf spot incited by *Cercospora zeae-maydis* is a new devastating foliar disease of maize in East Africa. For effective control, elucidation of the most critical elements of the grey leaf spot disease pyramid is important. This study investigated the role of mineral nutrition, pathogen variability and host resistance in the epidemic. Trials were conducted under field and controlled environments. The 28 isolates used in the controlled environment varied significantly ($P \leq 0.05$) in parasitic fitness measured indirectly as disease efficiency, but no infection pattern could be attributed to known *C. zeae-maydis* pathotypes. Data from field trials showed that host resistance and mineral nutrition significantly ($P \leq 0.05$) affected disease efficiency, with highest disease development occurring in nitrogen-augmented plots. Exclusive phosphorus application had no clear effect on grey leaf spot epidemics but combined application with nitrogen significantly ($P \leq 0.05$) reduced the predisposition effects of nitrogen to the disease. Overall, treated plots had less disease than unfertilised plots. Fertiliser application had no effect on sporulation capacity, while cultivars significantly affected it. Geographic differences in amount of disease were observed, suggesting environment influences on grey leaf spot incidence. The results suggest that the current grey leaf spot epidemics in East Africa are due to favourable cultivars, poor mineral nutrition and environmental interactions.

Introduction

Maize is Uganda's and East Africa's most important cereal. As a consequence, deliberate efforts to increase production by use of elite cultivars and fertilisers are being promoted by East African governments (GOU, 2000). However, a major obstacle to increased maize production in East Africa is a new foliar disease, grey leaf spot caused by *Cercospora zeae-maydis* Tehon and Daniels (Bigirwa et al., 1999; Okori et al., 2001; Asea et al., 2002). In the United States, reduced or non-tillage maize production system has increased grey leaf spot severity (de Nazareno et al., 1993). In Africa, where such a system is rare or absent, incidence and severity of grey leaf spot is similarly high. This is presumably due to continuous cropping of maize all year round in response to high demand and the relatively longer growing season of up to 300 days in Africa compared to 120–150 days in the US (Ward et al., 1999). Maize is intolerant to nutrient deficiency. Nevertheless, soil fertilisation is reported to influence grey leaf spot epidemics (Smith, 1989; Ward, 1996). Thus, it is clear that like other plant disease epidemics, grey leaf spot is a product of interactions between host, pathogen and environment as impacted upon by man (Zadoks and Schein, 1979). In the US, grey leaf spot has spread slowly, spanning over 50 years before reaching epidemic proportions (Latterell and Rossi, 1983; Ward et al., 1999). Whereas in Africa, the disease has spread faster since it was first reported in South Africa in 1990–1991 and is now endemic in most sub-Saharan countries (Ward and Nowell, 1998; Ward et al., 1999). There are, however, differences in the levels of disease severity between the two continents, being higher

in Africa than in the US (Asea and Adipala, 2001). Such differences in disease severity between the two continents suggest variation in the effects of grey leaf spot disease pyramid components. In the grey leaf spot pathosystem, pathogen variability has been suggested as a possible cause for increased severity in the US (Bair and Ayers, 1986). Studies on the parasite fitness of *C. zeae-maydis*, as measured using disease efficiency, indicted that 3 out of 15 isolates significantly accounted for variation in epidemics (Bair and Ayers, 1986). Recent population studies of *C. zeae-maydis* revealed the presence of two pathotypes designated group I and II (Wang et al., 1998). Group II, was more genetically diverse than group I, and predominant in Africa (Dunkle and Levy, 2000; Okori et al., 2001). Pathogen populations are typically composed of many different pathotypes or genotypes, with only a subset being most frequent accounting for epidemics (Burdon, 1993). In the case of *C. zeae-maydis,* no specific epidemic patterns in relation to the two pathotypes have been reported. However, investigations in the US show that different *C. zeae-maydis* populations vary in aggressiveness, in part, accounting for variation in epidemics (Carson et al., 2002). As such, variability in aggressiveness of group II isolates cannot be precluded as one of the factors influencing the current epidemics in East Africa. No similar studies have been conducted in sub-Saharan Africa where grey leaf spot epidemics incidentally clearly differ from the temperate regions (Asea and Adipala, 2001). Yet provision of such data would support breeding programmes and genotype deployment. Aggressiveness, an indicator of the parasitic fitness of a pathogen may be influenced besides the pathogen's innate pathogenicity, by interactive effects of other disease pyramid elements (Nelson, 1979). In the case of *C. zeae-maydis,* weather conditions and survival of the fungus in crop debris are reportedly critical (Jenco and Nutter, 1992). There is, however, limited information on interactive effects of other disease pyramid components such as host and mineral nutrition on parasitic fitness of the known *C. zeae-maydis* pathotypes and any role(s) that these may play in current epidemics in the tropics. Previous reports on the effects of mineral nutrition on grey leaf spot epidemics are contradictory and indicate the need for further investigations (Ward and Nowell, 1998). Hence, this study was performed to investigate: (i) the role of variability among group II isolates on grey leaf spot epidemics in the tropics and (ii) the interactive effects of mineral nutrition and host resistance on the pathogen's aggressiveness and disease development.

Materials and methods

Two trials were conducted to examine the interactive effect of elements affecting parasitic fitness of *C. zeae-maydis.* The first trial investigated the role of pathogen variability on grey leaf spot severity. It was performed in two stages by screening isolates for genetic variability using restriction fragment length polymorphism (RFLP) and subsequently using them to test for phenotypic (pathogenicity) variability on the host under controlled environments. The second trial studied the relative effect of mineral nutrition and host resistance on disease development under field conditions. Parasitic fitness was assessed based on aggressiveness, which in turn was assessed using disease efficiency, defined as severity of disease as indicated by percent leaf area affected and area under disease progress curve (AUDPC) from a given amount of inoculum, on a particular host genotype, under a given set of environmental conditions. Disease efficiency combines infection efficiency and virulence (Nelson, 1979), which were previously used as indicators of parasitic fitness in *C. zeae-maydis* and other pathogens (Bair and Ayers, 1986; Kadish and Cohen, 1988). Sporulation capacity, another component of parasitic fitness, was also measured.

Plant material. In the first trial, a popcorn maize cultivar (susceptible open pollinated local cultivar) was used. In the second trial, in addition, the cultivars Longe 1 (moderately resistant open pollinated cultivar) and SC 627 (resistant hybrid) were used.

Effect of pathogen variability on grey leaf spot

Test for genetic variability. Twenty-seven *C. zeae-maydis* monoconidial isolates out of 150 collected were selected as representatives from different geographical locations and used in the study (Table 1). Also included were four isolates from the US, GLS3 and GLS5 shown to belong to group II, GLS2 to group I (Okori et al., 2001) and GLS1 an isolate whose pathotype status as group II, was not known before the present investigation. All test isolates were examined for genetic variability using RFLP before subsequent use in pathogenicity trials. Fungal DNA was isolated accordingly (Möller et al., 1992; Kuusk et al., 2002) and Southern blot and hybridisations were performed following the procedures for Hybond N+ nylon membranes (Amersham Phamarcia AB, Uppsala, Sweden).

Table 1. Origin and designations of isolates used in the study

Isolate	Origin	Location[a]
Ug3	Masindi, Uganda (i)	West
Ug4	Tingei, Kapchowra, Uganda	East
Ug5	Samazi, Mbale	East
Ug6	Ikulwe, Iganga, Uganda	East
Ug9	Musita, Iganga, Uganda	East
Ug10	Jinja, Uganda (i)	East
Ug13	Kyabakuza, Masaka, Uganda (i)	South-west
Ug14	Kyabakuza, Masaka, Uganda (ii)	South-west
Ug15	Kyazanga, Masaka, Uganda	South-west
Ug27	Jinja, Uganda (i)	East
Ug29	Nyendo, Masaka, Uganda	South-west
Ug30	Masindi, Uganda (ii)	South-west
Ug31	Mbale, Uganda	East
Ug32	Buyaga, Mbale, Uganda	East
Ug33	Kamemyamigo, Masaka, Uganda	South-west
Ug39	Samazi, Mbale	East
Rw17	Butare, Rwanda	South
Rw18	Cyangugu, Rwanda (i)	West
Rw19	Cyangugu, Rwanda (ii)	West
Rw23	Kigali, Rwanda	East
CZm2a	Kabanyolo, Mpigi, Uganda (i)	Central
CZm2b	Kabanyolo, Mpigi, Uganda (ii)	Central
CZm3b	Kabanyolo, Mpigi, Uganda (iv)	Central
CZm4a	Kabanyolo, Mpigi, Uganda (v)	Central
CZm6a	Kabanyolo, Mpigi, Uganda (ix)	Central
CZm6b	Kabanyolo, Mpigi, Uganda (ix)	Central
GLS1	Aberdeen, Maryland, USA	East
GLS2[b]	IIinois, USA	East
GLS3[c]	Davidson, N. Carolina USA	East
GLS5[c]	Indiana, USA	East

[a]Locations are with respect to the countries of origin. (i), (ii), (iii), (iv), (v), and (ix) designate isolates collected from different fields in the same location.
[b]GLS2 belongs to group I (Okori et al., 2001).
[c]GLS3 and GLS5 belong to group II (Okori et al., 2001).

A genomic library was made using the isolate CZm2a. Fungal DNA and pUC18 plasmid vector DNA was digested to completion using *Eco*RI, ligated and transformed into the *Escherichia coli* DH5α strain and subsequently used as probes for RFLP analysis (Sambrook et al., 1989). Each probe $\times$ restriction enzyme combination was treated as a RFLP locus and the restriction size variants detected by each probe treated as alleles at that locus. Alleles at different loci were first screened individually and later combined to constitute multilocus RFLP genotypes, which were used for the final analysis. The data was also subjected to phenetic analysis using Saitou and Nei's Neighbour joining method with 500 permutations to test for robustness of the tree using Phylip (Felsenstein, 1999). The consensus phenetic tree was drawn using TreeView version 1.6.1 © 2000 Roderic D.M. Page.

Test for variability in pathogenicity. The isolates analysed for polymorphism using RFLP were subsequently used to test the role of pathogen variability on grey leaf spot severity. Trials were conducted in a culture room and greenhouse. Seedlings of popcorn were used for both experiments. For the culture room trial, seeds were surface sterilised before plating on sterile solid media (Steventon et al., 2002). Before use in this study, each isolate was re-introduced to its host under *in vitro* conditions (Steventon et al., 2002) and isolated from sporulating lesions to maintain pathogenicity. Inoculum was then prepared from these fresh cultures. After 10 days of growth, the fungal cultures were homogenised for 30 s using a Waring blender, poured to fresh V8 juice plates and incubated for 9 days in darkness and for 3 days under constant light to induce sporulation (Beckman and Payne, 1982). Spores were harvested using a 0.01% Tween 20 solution and the spore concentration adjusted to 1.6×10^4 spores ml^{-1}. The spore suspension was used directly to inoculate seedlings at the two-leaf stage, V2 (Ritchie et al., 1993), by applying 200 μl of the spore suspension into leaf whorls. Inoculated seedlings were transferred to a culture room under 16 h light at 22 °C and 8 h darkness at 16 °C where infection and development of the disease was observed. The trial was arranged following a randomised complete block design, with two blocks, each consisting of five test plants inoculated with each isolate. The greenhouse trial was set up as the one in the culture room, except that the plants were grown in 150 g of a sterilised commercial premixed potting soil (K-jord, Svalöf–Weibull, AB, Sweden). Inoculation was done at the four-leaf stage (V4) by brushing leaf surfaces with spore suspension. Thereafter, each plant was covered with a plastic bag during the entire study period both to raise the relative humidity and to mimic mist or dew (Beckman and Payne, 1983). The plastic bags also provided isolation barriers between different isolates.

Effects of host resistance and mineral nutrition on grey leaf spot

These trials aimed at elucidating relative effects of mineral nutrition and host resistance on disease development under field conditions. They were conducted at two grey leaf spot endemic locations with different ecological conditions to measure environmental

effects on the disease. The first site was in the wet-humid lake Victoria basin of Uganda, at Makerere University Agricultural Research Institute, Kabanyolo (MUARIK). Trials started in September 1999 and lasted to January 2000 and were repeated in two successive cropping seasons, i.e. March–July 2000 and August 2000–January 2001. The second field site was in the drier south-western Uganda, at Masaka District Farm Institute, Kamemyamigo, (Masaka DFI) starting similarly in the September–January 1999 cropping season and repeated once from March to July 2000. Prior to establishing the trials, soil analysis was performed at Department of Soil Science, Faculty of Agriculture, Makerere University, on field samples from both sites to determine the nutrient status of the plots. Total nitrogen (N) and available phosphorus (P) were determined together with exchangeable potassium (K), soil pH and texture (Okalebo et al., 1993; Anonymous, 1996). The results showed that the soils at both sites were deficient in nitrogen (0.18% and 0.14%) and available phosphorus (4.49 and 4.17 ppm). While potassium at 0.31 milliequivalent/100 g soil and 0.28 milliequivalent/100 g soil was above the threshold of 0.2 milliequivalent/100 g soil, suggesting sufficient K quantity in the soil (Landon, 1991). This information was used to compute N and P application rates corresponding to high and recommended levels of 100 and 60 kg ha^{-1} P and 240 and 72 kg ha^{-1} N. Based on the high and low rates, seven nutrient regimes of N_1 = recommended rate, N_2 = high rate, P_1 = recommended rate, P_2 = high rate, their combinations N_1 P_1, N_2 P_2 and a non-fertilised control were formulated. Nitrogen was applied in form of granular urea and P as single super phosphate. Nutrient regimes were designed to investigate earlier reports of the three macronutrients (NPK) either positively influencing grey leaf spot epidemics (Smith, 1989: Ward, 1996), or not having any effect at all (Smith, 1989; Carrera and Grybauskas, 1992). All the three maize cultivars were subjected to the seven nutrient regimes. Plants were established in two row plots, 4 m long, at a spacing of 75 cm between rows and 25 cm between plants following a randomised complete block design with three replications. Two rows of popcorn were planted between test cultivars to increase disease pressure. Fertilisers were applied by side dressing, and incorporated into the soil when the plants were between growth stages V7 and V8.

Inoculum preparation. Each of the two experimental sites was treated as an epidemiological unit consisting of a given population of *C. zeae-maydis*. Even in places where grey leaf spot was endemic, disease development is largely dependent on inoculum build-up usually from crop debris or continuous cropping (de Nazareno et al., 1993; Asea et al., 2002). In this study, the experimental sites had not been under maize the previous season, and therefore, to a large extent, the effect of natural inoculum were minimised. It was thus necessary to artificially inoculate the plants to increase disease pressure. In the first cropping season, test plants at each experimental site were inoculated with isolates originally collected from maize fields in the same locality. In the case of Masaka DFI, inoculum was prepared from the isolates Ug13 and Ug33 while in the case of MUARIK, CZm2a and CZm2b were used. The rationale for this manner of inoculum use was to increase the population frequencies of test isolates at the experimental sites to permit comparison of field and controlled environment data. Inoculum was prepared from monoconidial cultures of the isolates maintained on potato dextrose agar plates. Cultures were inoculated on sterile sorghum seed contained in plastic bags and mixed manually every 3 days to permit even fungal colonisation of sorghum kernels for 10 days. Subsequent inoculum was prepared from monoconidial cultures obtained from lesions collected from the plants inoculated with the test isolates in the first cropping season of experimentation. Before inoculation, the sorghum kernels were air dried and directly used to inoculate the maize plants by placing about 20 colonised kernels in the leaf whorls at plant growth stage V6.

Disease assessment and data analysis. Disease assessment commenced 11, 14 and 18 days after inoculation in the culture room, greenhouse and field experiments, respectively. In the field trials, disease rating was performed using a 0–50% scale on whole plant basis for five consecutive weeks (Ward et al., 1997). In the culture room experiments, the disease rating scale of Ward et al. (1997) was modified to suit small plants as: 2 = no blight but with 1–3 flecks, 5 = 2–4 lesions on the first two leaves, 10 = larger lesions on first three leaves (about 10% infection), 20 = about a quarter of seedling leaves showing symptoms (20% blighted), 35 = over one-third of plant blighted with lesion coalescence (35% infection), 50 = 50% of the plant tissues blighted. Severity ratings were taken weekly and at least five recordings were taken per experiment. Sporulation capacity, measured as the number of conidia produced per square centimetre of lesion was quantified from lesions collected 42 days after inoculation

in the field trial at MUARIK. This was to test an earlier observation where no significant differences in sporulation capacity of isolates had been observed.

Weekly disease severity data from all experiments were used to calculate AUDPC and the value was standardised by dividing it by the total number of days of the epidemic (Campbell and Madden, 1990). Final severity ratings, AUDPC and sporulation capacity were subjected to one and two factor analysis of variation (ANOVA) for controlled and field experiments, respectively. Where significant differences were found at $P \leq 0.05$, means were compared using Fisher's protected least significant difference test (LSD) (Steel et al., 1997). Prior to ANOVA, spore data was transformed using ln(spores + 1) to normalise variance. Tests for variance homogeneity between experimental sites or successive repetitions were done using Fisher's or Bartlett's tests, appropriately (Steel et al., 1997). When no homogeneity was found, combined data analysis was omitted. Analysis was performed using Genstat 5 version 3.2 1995 (Lawes Agricultural Trust: Rothamsted Experimental Station, UK) and MINITAB 13.31(Minitab Inc, Pennsylvania, USA).

Results

Effect of pathogen variability on grey leaf spot

Test for genetic variability. Phenetic analysis based on RFLP data confirmed that 26 East African originated isolates belonged to group II with only one isolate (Ug4) clustering with a US group I isolate (Figure 1). The group II cluster had several sub-clusters supported by bootstrap values of over 95. Within the sub-clusters, isolates were similar with short genetic distances between isolates.

Test for variability in pathogenicity. In the greenhouse and culture room trials, the inoculated plants exhibited the characteristic grey leaf spot rectangular lesions 10–14 days after inoculation. The data were not combined because of absence of variance homogeneity between experiments. Disease efficiency as measured by AUDPC and final severity varied significantly ($P \leq 0.05$) (Table 2). On the basis of AUDPC, only three group II isolates Ug30, Ug5 and Ug39 consistently had a higher disease efficiency than GLS2 (group I pathotype) in both trials (Table 2). Although the rest of the group II (24) isolates had varied disease efficiency as measured using AUDPC, under the two experimental conditions. Final severity data showed a similar trend

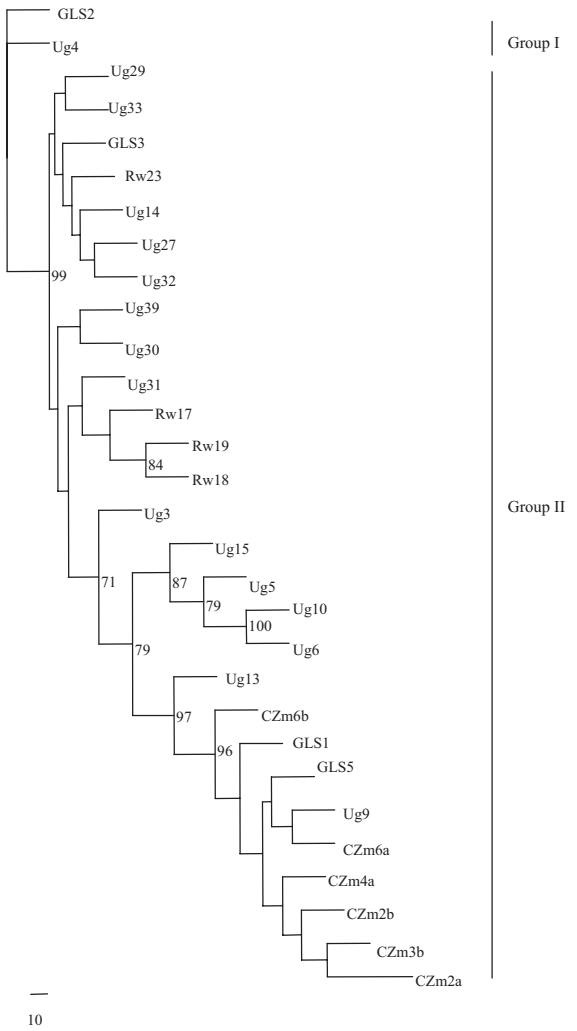

Figure 1. Neighbour-joining dendrogram of RFLP based on 600 polymorphic fragments of 30 *Cercospora zeae-maydis* isolates. Numbers at the nodes are bootstrap values greater than 70 generated from 500 permutations. The consensus tree was computed using the majority-rule consensus tree method of Phylip (Felsentein, 1999). Branch lengths are proportional to the genetic distance, as indicated by the bar.

to AUDPC, with some group II isolates causing more disease than the group I isolate GLS2, particularly, under culture room conditions where more disease developed (Table 2). The US group II isolates were not significantly different in their disease efficiency from each other and in general, the majority of the group II isolates (African and American) did not significantly differ in their disease efficiency. Correlation between AUDPC values for greenhouse and culture room experiments though significant ($P \leq 0.05$), was

Table 2. Final severity and AUDPCs of grey leaf spot on a susceptible maize cultivar (popcorn) when inoculated with *C. zeae-maydis* isolates under greenhouse and culture room conditions

Isolate	Greenhouse[a]		Culture room[b]	
	Final severity (%)	AUDPC[c]	Final severity (%)	AUDPC[c]
Ug3	11.00	28.55	25.00	28.55
Ug4	5.50	28.52	5.67	2.39
Ug5	27.50	42.88	35.00	30.09
Ug6	31.65	35.17	—	—
Ug9	22.5	33.25	20.00	9.46
Ug10	20.00	35.00	20.00	20.42
Ug14	23.75	36.22	25.00	14.60
Ug15	17.50	37.62	40.00	38.69
Rw17	21.25	45.32	30.00	15.33
Rw18	29.55	37.45	18.33	21.56
Rw19	26.25	35.00	25.00	16.15
Rw23	21.25	39.02	18.33	14.19
Ug27	23.75	33.78	13.33	10.32
Ug30	23.75	39.90	30.00	26.83
Ug31	21.85	32.90	35.00	21.20
Ug32	15.65	34.12	21.67	10.87
Ug33	27.50	44.27	16.67	14.98
Ug39	26.25	47.95	35.00	26.94
GLS1	25.00	35.87	31.67	20.57
GLS2	20.25	29.75	10.00	15.60
GLS3	11.75	29.75	25.00	16.89
GLS5	12.50	28.00	—	—
CZm2a	20.00	23.80	16.67	13.08
CZm2b	30.00	28.00	30.00	22.45
CZm3a	27.50	31.50	—	—
CZm3b	35.00	36.75	20.00	19.44
CZm4a	10.00	25.02	20.00	10.68
CZm6a	10.00	28.00	13.33	10.68
LSD	13.84	10.10	11.04	9.74
CV%	23.8	5.5	11.30	10.10

[a]Inoculated with 28 isolates.
[b]Inoculated with 25 isolates.
[c]Calculated according to Campbell and Madden (1990). Means were compared using Fisher's protected LSD test at $P \leq 0.05$.

not strong ($r^2 = 0.50$). Overall, no obvious pathogenicity pattern could be attributed to pathotype or isolates from specific geographic origin.

Effects of host resistance and mineral nutrition on grey leaf spot

AUDPC and final disease severity were used as estimates of disease efficiency for field populations of *C. zeae-maydis*. The data show that fertiliser regimes significantly ($P \leq 0.05$) affected disease progress as measured by AUDPC at both locations, in all cropping seasons (Table 3). At MUARIK, the cultivars differed significantly ($P \leq 0.05$) in their disease reaction and the interactive effects of the two factors (cultivar × fertiliser regimes), significantly ($P \leq 0.05$) affected disease development (Table 3). The most resistant cultivar SC 627 had the lowest disease level, while popcorn, the most susceptible genotype, had the highest disease level (Figure 2). Non-fertilised control plants developed the highest disease levels throughout the experimental period on the moderately resistant Longe 1 and the susceptible popcorn. In general, separated means revealed that plots subjected to only nitrogen application (both high and low rates) had highest disease levels compared to other fertilised plots. No clear pattern could however, be attributed to high or low fertiliser application rates, although, in general, high N rates increased disposition to grey leaf spot disease (Table 3). The effect of exclusive P application on disease efficiency was not significant for most seasons when compared with the control plots. Combined application of N and P especially at recommended levels (N_1P_1), significantly ($P \leq 0.05$) reduced the predisposing effects of high N application during all the three cropping seasons at MUARIK (Figure 2). Furthermore, ANOVA revealed no significant effects of fertiliser regimes on sporulation capacity of the fungus. Only cultivar effects significantly ($P \leq 0.05$) affected sporulation capacity.

At the second location, Masaka DFI, results were similar to those in the first location (MUARIK). AUDPC and final severity were significantly ($P \leq 0.05$) affected by fertiliser regimes during the two cropping seasons (Table 3). Cultivar × fertiliser interactions were similar to the MUARIK data (Figure 2). Disease severity varied with cropping season although no clear pattern was evident at both locations, indicative of responsiveness of *C. zeae-maydis* to weather changes each cropping season.

Discussion

The present investigation sought to elucidate factors accounting for the current epidemics of grey leaf spot in East Africa using multiple approaches. Investigations into the possible role of differential fitness among the isolates revealed no clear association between pathotype (groups I and II) and disease severity. However within group II, significant differences in disease

Table 3. Mean values of three test cultivars Longe 1, Popcorn and SC 627 showing effects of different fertiliser application regimes on sporulation capacity, AUDPCs and final severity of grey leaf spot at MUARIK and Masaka DFI

Treatment	AUDPC[a]					Final severity (%)[b]					Sporulation capacity[d,e,f]
	MUARIK			Masaka DFI		MUARIK: Cropping seasons			Masaka DFI		MUARIK
	1[c]	2	3	1	2	1	2	3	1	2	1
N_1	9.58	11.25	9.64	18.06	3.66	8.80	13.40	14.50	13.10	4.90	13.30
N_2	8.62	9.59	9.00	17.91	2.87	9.60	13.10	15.80	13.10	6.80	13.20
P_1	6.63	8.21	6.49	10.26	2.25	7.40	7.80	15.20	13.80	4.60	13.70
P_2	9.67.	11.89	7.15	13.48	3.74	9.40	9.90	14.00	11.60	5.90	13.30
N_1P_1	9.89	10.60	8.01	15.54	4.27	7.20	9.10	12.50	6.70	2.70	13.90
N_2 P_2	6.10	8.67	8.50	13.54	2.37	5.80	8.26	16.80	9.90	4.20	13.60
Control	10.98	14.28	9.38	17.16	5.16	11.60	17.40	16.70	12.50	6.80	13.20
LSD	1.96	3.00	2.34	4.99	1.88	2.50s	4.60	3.80	4.06	NS	NS
CV %	10.00	10.60	12.40	6.70	15.50	7.20	15.30	6.30	17.90	19.40	30

N_1 = application at recommended rate of 72 kg N ha^{-1}, N_2 = application at a rate of 240 kg N ha^{-1}, P_1 = application at recommended rate of 60 kg P ha^{-1}, P_2 = application at a rate of 100 kg P ha^{-1}, N_1 P_1, N_2 P_2 = combinations of the two nutrients.

[a]Calculated according to Campbell and Madden (1990).

[b]Final severity (% plant leaf area affected) taken 49 days after inoculation.

[c]1 = September 1999–January 2000, 2 = March–July 2000, 3 = August 2000–January 2001.

[d]Samples taken 42 days after inoculation from 1 cm^2 of lesion area. [e]Spore counts transformed by ln(spores + 1).

[f]Samples taken only in the first cropping season.

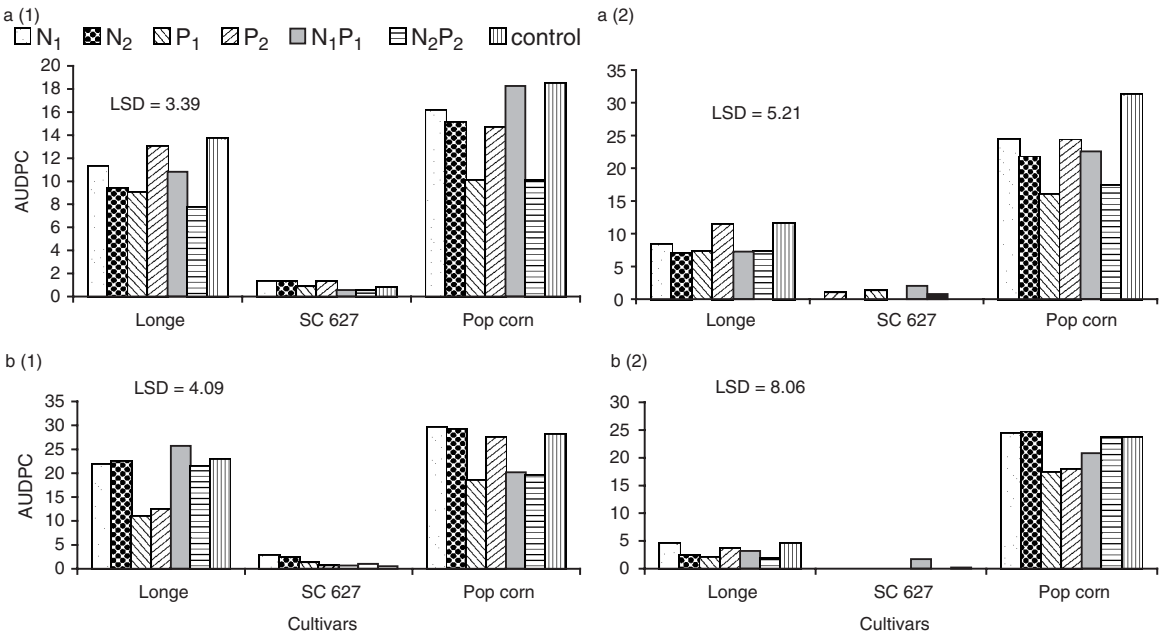

Figure 2. Cultivar by fertiliser regime interaction effect on grey leaf spot development at two sites, MUARIK (a) and Masaka DFI (b) in Uganda during first two cropping seasons September–January 1999 (1) and March–July 2000 (2) of experimentation. AUDPC was computed according to Campbell and Madden (1990). Means were compared using Fisher's protected LSD test at $P \leq 0.05$. Fertiliser application rates were $N_1 = 72\,\text{kg ha}^{-1}$, $N_2 = 240\,\text{kg ha}^{-1}$, $P_1 = 60\,\text{kg ha}^{-1}$, $P_2 = 100\,\text{kg ha}^{-1}$.

efficiency were found. This suggests that differences in disease severity observed in the field could be attributed to isolate aggressiveness. Our observations in the field indicate that disease severity is higher in some locations than others. Similar results have been reported in the US for *C. zeae-maydis* (Bair and Ayers, 1986; Carson et al., 2002), emphasising the need for multi-location variety evaluation in East Africa. Parallel observations have been made on *Exserohilum turcicum,* another foliar disease of maize endemic in Eastern and Southern Africa (Adipala et al., 1993). Thus, it appears that East African populations of *C. zeae-maydis* comprise one epidemiological unit derived from a common gene pool, but whose disease efficiency is largely regulated by epidemiological factors. This study shows that the resistant variety SC 627 markedly reduced *C. zeae-maydis* disease efficiency, in contrast to the susceptible variety where disease efficiency was high. The significant increase in sporulation capacity dependent on cultivar resistance and not on isolate differences, provides evidence for the primary role of host resistance in the current epidemics. On the whole, it appears that the East African *C. zeae-maydis* population when treated as one epidemiological unit, consists of a mixture of several genotypes. The low selection pressure (few resistant lines deployed) permits general multiplication of several *C. zeae-maydis* haplotypes. This increases disease severity and incidence by provision of haplotypes with diverse fitness. Within season, spread apparently depends on severity, while successive maintenance of populations relies on ability to survive periods of host absence (Stromberg, 1986; de Nazareno et al., 1993). Once deployed, resistant lines would raise the selection pressure, dramatically eliminating less fit *C. zeae-maydis* genotypes. Therefore, host resistance is the choice control tool since it can slow grey leaf spot epidemics by reducing inoculum pressure and efficiency. Moreover, resistance is widely viewed as environmentally friendly and economically manageable by resource constrained farmers (Ward and Nowell, 1998; Ward et al., 1999). Freppon et al. (1994) have suggested that selection of resistant lines to grey leaf spot should be based on low severity, targeting reduced lesion size and numbers, since these factors would impact on sporulation capacity and other parasitic fitness attributes. For reliable evaluation, testing should be conducted at multi-locations. Recent studies show that genotype by environment interactions, a problem encountered during field evaluations for grey leaf spot resistance, may be explained in part by variety by isolate interactions and could easily be managed through wider regional testing (Carson et al., 2002).

The other crucial factor influencing epidemics in East Africa is host nutrition. Disease efficiency of *C. zeae-maydis* in this study was affected by host nutrition and resistance status. Poor host nutrition in form of deficiency or unbalanced applications of macronutrients predisposed plants to grey leaf spot in agreement with earlier reports (Smith, 1989; Ward, 1996). This indicates that in East Africa, where the soils are heavily farmed without fertilisation, poor nutrition, in part, accounts for the high disease severity especially when susceptible cultivars are used. Nitrogen application predisposed plants to the disease perhaps due to disruption of its normal phytohormone system (Marschner, 1986). This differs from an earlier report, which indicated that nitrogen application to maize had no effects on grey leaf spot in Maryland, USA (Carrera and Grybauskas, 1992). Conversely, exclusive phosphorus application had no clear effect on grey leaf spot development as was similarly reported by Smith (1989). This synergistic effect of nitrogen and phosphorus in reducing *C. zeae-maydis* disease efficiency could be due to favourable changes in the phytohormone balance, given that, phosphorus availability enhances nitrogen metabolism (Landon, 1991). Severe nitrogen and phosphorus deficiencies are common in tropical arable land. Thus combined remedial applications can ameliorate grey leaf spot epidemics. The absence of clear effects of excess macronutrients in this study demonstrates that application rates above remedial levels, carry no advantages as suggested by Liebig's law of minimum (Waggoner and Norvell, 1979).

This study has attempted to identify the most crucial elements of the grey leaf spot disease pyramid in East Africa accounting for the current epidemics. The data shows that *C. zeae-maydis* epidemiological efficiency in the tropics is influenced extensively by host factors such as susceptibility and poor mineral nutrition. The combined effects of these factors and the polycyclic nature of the pathogen generate a high inoculum pressure each cropping season. Additionally, differences in fitness of *C. zeae-maydis* haplotypes would also be influenced by the above-mentioned factors. As such, increased fertiliser application especially nitrogen, in maize production, as currently advocated by many sub-Saharan governments, may in fact increase epidemics if not well managed. Nevertheless, if well managed, especially under an integrated disease management

framework, sustained production is possible without exerting extreme selection pressure on the pathogen resulting in development of novel pathogen genotypes. Deployment of resistant lines in combination with appropriate nutrition and other cultural practices are suggested suitable tools that could reduce inoculum pressure, especially for resource-constrained East African farmers.

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