

**A STUDY OF *CERCOSPORA ZEA-MAYDIS* ON MAIZE (*ZEA MAYS* L.) IN
RELATION TO ITS VARIABILITY AND CONTROL IN ZAMBIA.**

BY

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**A DISSERTATION SUBMITTED TO THE UNIVERSITY OF ZAMBIA IN
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MASTER OF SCIENCE IN PLANT PATHOLOGY**

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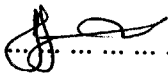
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DECLARATION

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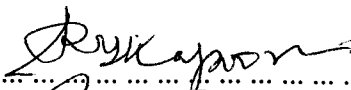
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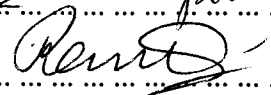
This dissertation of FLORENCE HANYUMA is approved as fulfilling part of the requirements for the award of the degree of Master of Science in Plant Pathology of the University of Zambia.

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Internal Examiners

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External Examiner

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DEDICATION

To my boys, Eric and Rene, for their endurance and patience throughout the course of this study.

ABSTRACT

Maize (*Zea mays* L.) is an important crop in Zambia but it is being threatened by a number of foliar diseases. In Zambia, gray leaf spot caused by *Cercospora zeae-maydis* has become a serious foliar disease of maize in the last four years.

The assessment of the variability of *Cercospora zeae-maydis* was done by taking samples from the three agro-ecological regions of Zambia. Samples were collected from Livingstone, Choma, Kabwe and Ndola. Four fields, which had natural infection of *Cercospora zeae-maydis*, were randomly sampled for each site. Diseased leaves were collected from the plants when the crop was nearing physiological maturity.

Sections of the diseased maize leaves were incubated to allow for the growth of the pathogen. Measurements of the conidial size were done after five days of incubation at 25 °C. An analysis of variance of size of conidia revealed significant differences within and between the agro-ecological regions of Zambia. The variability observed could be attributed to the differences in the climatic conditions in the agro-ecological regions.

A susceptible variety, SC 401, was used to assess the effectiveness of Early Impact fungicide (150g carbendazim and 94g flutriafol per litre) on the control of gray leaf spot disease on maize. The experimental sites used were Mt. Makulu Research Station and University of Zambia School of Agriculture field station. Three treatments and a control were used. The experiments were replicated three times in a Completely Randomised Design.

Spraying commenced at 62 days after emergence of the maize crop when the disease index was 35. A second spray was applied 21 days after the first fungicide application. Disease index increased on the control plots. For those that received a single fungicide application, the disease index decreased initially but increased again subsequently. Those that received two or three fungicide applications demonstrated a decrease in the disease index throughout the experimental period.

Early Impact can effectively control gray leaf spot disease on maize with an economic benefit of 425% if a single fungicide application is made at the appropriate time. However, if the disease pressure is high, two fungicide applications would be necessary to control gray leaf spot on maize.

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LIST OF ABBREVIATIONS

Cm ³	Cubic centimetre
FAO	Food and Agricultural Organisation
GLS	Gray leaf spot
GRZ	Government of the Republic of Zambia
K	Zambian Kwacha
Kg	Kilogramme
ha	Hectare
L	Litre
LSD	Least Significant Difference
m	Metre
mm	Millimetre
MtM	Mt. Makulu Research Station
µm	Micrometre
Unza	University of Zambia

1.0 INTRODUCTION

Maize (*Zea mays* L.) is the third most important world crop after wheat and rice. It is believed to have originated from Mexico, and spread from there to other parts of the world. The spread was due to the development of the new world market for slave trade that required a cheaper source of energy, which could be easily transported and stored (Miracle, 1966; Sprague, 1977).

Maize has been grown in Zambia since colonial rule. It is utilised by about 80% of the population as a staple food. It is used as livestock feed and also has a number of other domestic uses, including its wide use in brewing. Maize contains about 5 - 6% proteins, 4.5 - 7% fats, and 76 - 88% carbohydrates (Miracle, 1966; Sprague, 1977). It is therefore the major source of energy for the Zambian population.

Maize is widely grown in Zambia especially along the line of rail. It requires a rainfall of at least 375 mm per growing season. The favourable temperature range for growth of maize is between 25-30 °C during the growing season (Ristanovic *et al.*, 1993). The soil pH requirements of maize range from 5 to 7.

Maize goes through four - grain filling stages, which are blister, milk, soft-dough, and hard-dough stages (Kling, 1991). It can be categorised into three kinds of varieties according to the period it takes to reach physiological maturity. These are early, medium and late maturing varieties.

Maize production in Africa and Zambia is threatened by a number of problems (CIMMYT, 1990). Major among these problems are diseases. This is because the environmental conditions that favour maize development also favour the development of diseases (Latterell and Rossi, 1983). Diseases hamper the development of the maize plant and reduce yield. Some of the major diseases of maize include cob rots and leaf blights. Gray leaf spot, caused by *Cercospora zeae-maydis*, has become a serious problem of maize during the last 3-4 years in Zambia (Nowell, 1997; Ward, *et al.*, 1999; Kaula – personal communication).

Cercospora leaf spot induced by *Cercospora zeae-maydis* Tehon and Daniels was first reported from Mkushi in 1995 and since then the disease has become a major problem in Zambia. It has now been reported from most regions of the country except those that receive less than 800 mm of rainfall per growing season. Gray leaf spot disease has been reported from most parts of Southern Africa, with the exception of Angola, Botswana, Lesotho, Mozambique and Namibia. It is widespread in South Africa, Zimbabwe, Malawi and Zambia (Ward *et al.*, 1997a; Nowell, 1997; Mushingo, 1999).

Gray leaf spot is characterised by elongated lesions delimited by veins. The lesions are gray to tan in colour and may be 0.5 to 5 cm long and 2 to 3 mm wide (Fig. 1). The lesions may coalesce, turn necrotic and cause early drying of the leaves. The disease is observed generally late in the growing season and particularly after the tasseling stage.



Fig. 1: Symptoms of gray leaf spot of maize caused by *Cercospora zeae-maydis*.

Cercospora zae-maydis produces conidiophores in stomatal tissue in clusters of about 20 (Latterell and Rossi, 1983; McGee, 1988). The conidiophores are dark brown in appearance and bear conidia sympodially (fig.2). The conidia are hyaline and have 3 to 10 septa (Shurtleff, 1973). They are wide at the base and taper at the apex (fig.3a and b). Mature conidia measure between 50 to 180 μm in length and 5 to 9 μm in width (Tehon and Daniels, 1925; Chupp, 1953; Kingsland, 1963; Latterell and Rossi, 1983). The size of conidia varies with conditions under which they are formed (Latterell and Rossi, 1983; Ward *et al.*, 1997b). The pathogen survives as mycelium in plant debris and has been found to survive for at least one year (Payne and Waldron, 1983; Beckman and Payne 1983; Latterell and Rossi, 1983).

Zambia is divided into three agro-ecological regions (Fig. 4). Region I in the south receive rainfall of not more than 800 mm per growing season. Region II occurring about the central parts of the country in the east-west trend, receives rainfall of between 800 to 1000 mm. Region III in the north receives rainfall of more than 1000 mm per growing season. This difference in the rainfall implies that each region differs in the amount of relative humidity and average temperature. It has been shown by Bair and Ayers (1986) that climatic variation in some regions can cause changes in spore morphology. In other regions climatic changes have no effect on spore morphology. Bair and Ayers (1986) stated that pathogen variability could be assessed on the basis of lesion and conidial size. The effect of climatic differences on conidial morphology will be examined in this study.

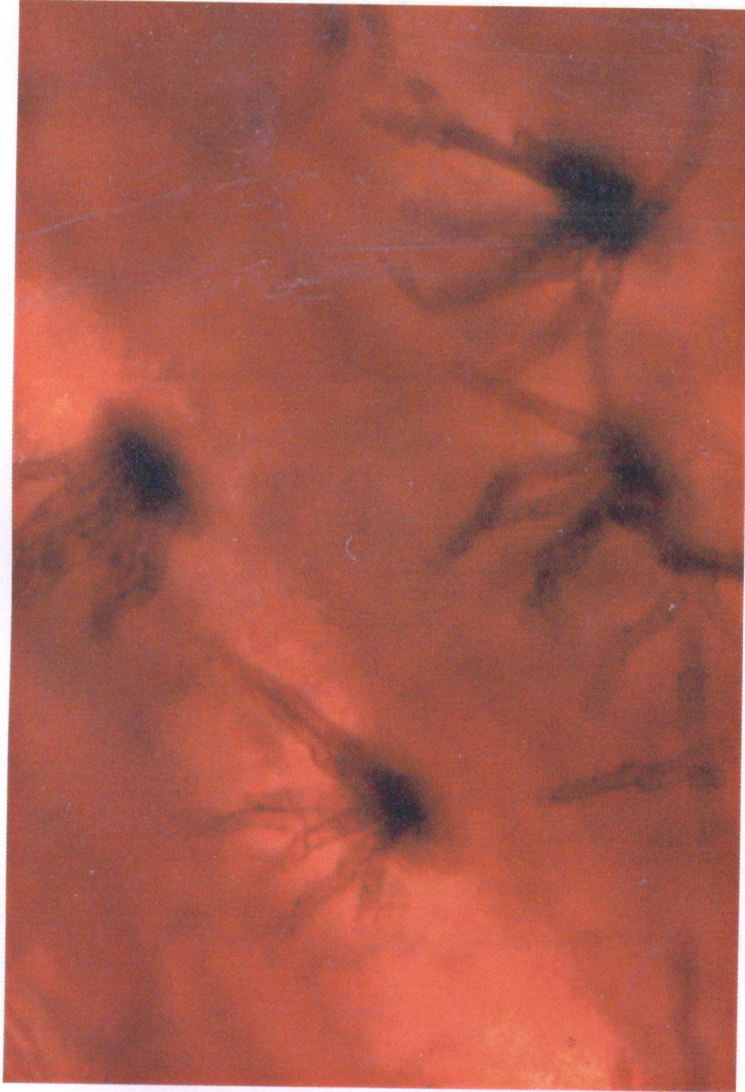


Fig. 2: Photomicrograph of *Cercospora zeae-maydis* showing clusters of conidiophores.

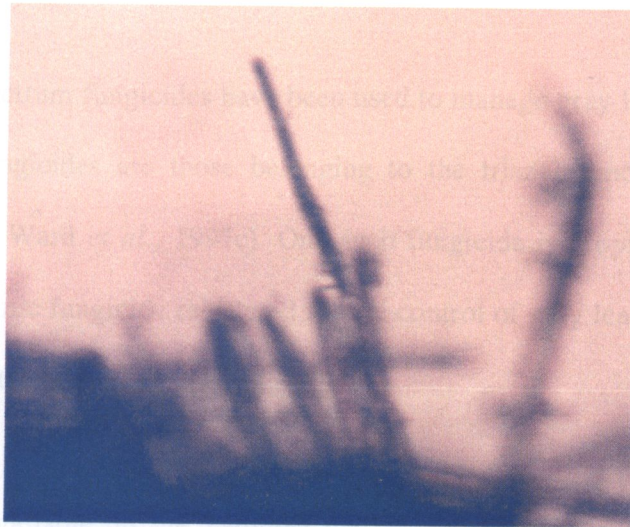


Fig. 3 a: Conidiophores of *Cercospora zeae-maydis* on maize leaf with one showing an attached conidium (x400)

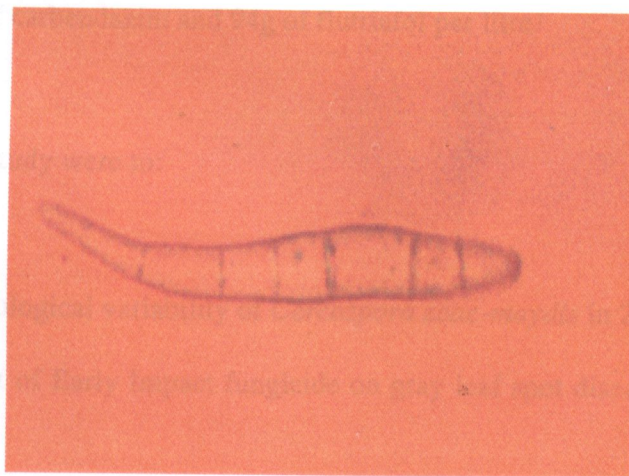


Fig. 3b: A single conidium of *Cercospora zeae-maydis* (x200)

A number of broad-spectrum fungicides have been used to manage gray leaf spot disease. The most effective fungicides are those belonging to the triazole and benzimidazole groups (Nowell, 1997; Ward *et al.*, 1997c). One such fungicide is propiconazole (Tilt), which is the only systemic fungicide registered for the control of gray leaf spot disease of maize in the United States of America (Jardine, 1999).

Some fungicides are available on the Zambian market and have been used to manage gray leaf spot although they are not registered for the control of gray leaf spot disease in Zambia. There is therefore a need to carry out a study on the effectiveness of the available fungicides. This study looked at the effectiveness of Early Impact fungicide, which contains 150g of carbendazim and 94g of flutriafol per litre.

The objectives of this study were to:

- examine the morphological variability of *Cercospora zeae-maydis* in Zambia,
- determine the effect of Early Impact fungicide on gray leaf spot disease development in maize,
- determine the effect of Early Impact fungicide on maize yield and,
- assess the financial benefits derived from the application of Early Impact fungicide.

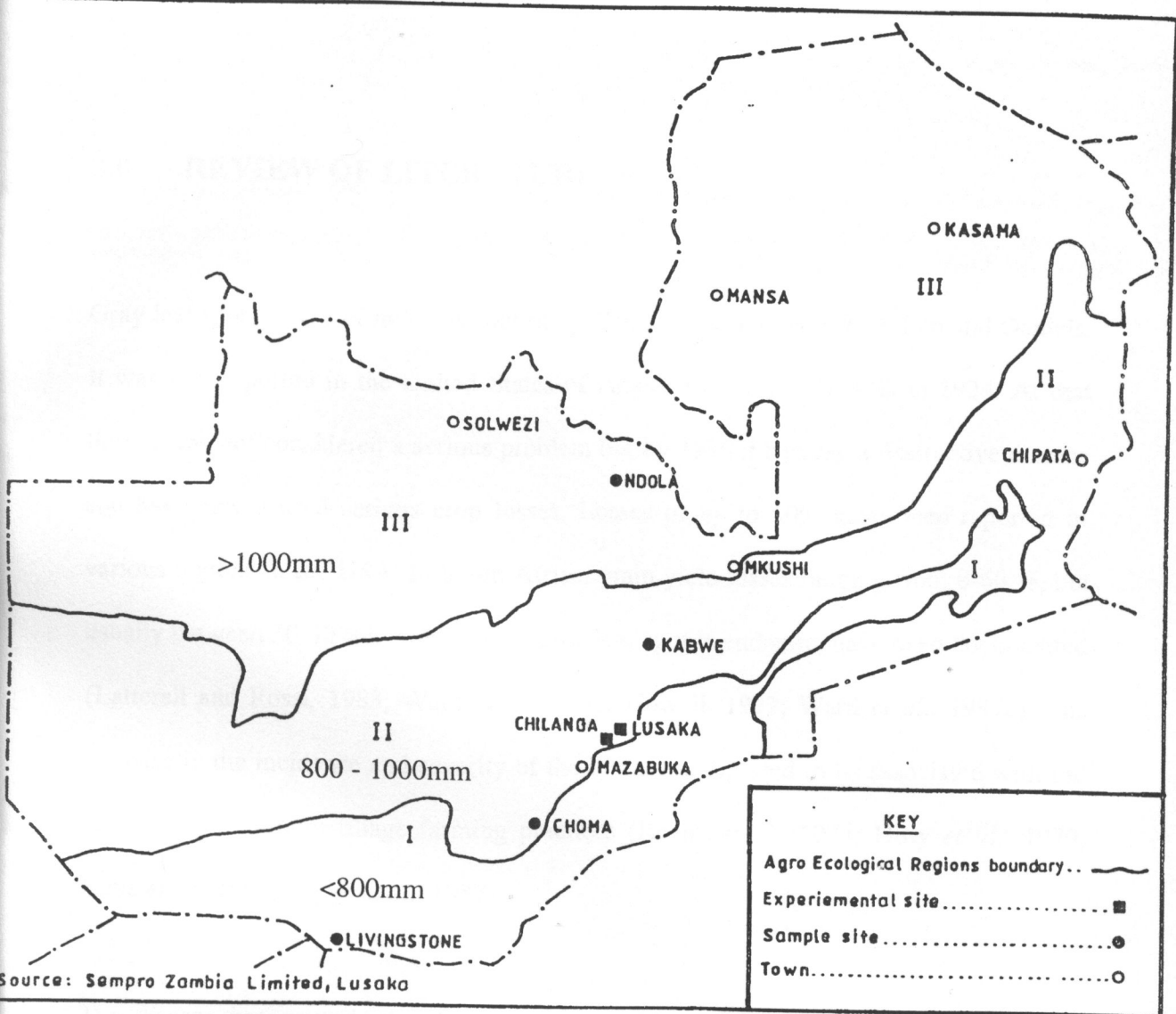


Fig. 4: Agro-ecological regions of Zambia.

2.0 REVIEW OF LITERATURE

Gray leaf spot disease of maize is caused by *Cercospora zeae-maydis* Tehon and Daniels. It was first reported in the United States of America (USA) in Illinois in 1924. At that time it was not considered a serious problem but by 1970 it became a destructive disease and has since caused serious crop losses. Losses of up to 50% have been reported in various regions in the USA. In South Africa, grain yield losses ranging from 0-60 %, but usually between 30-40% in areas where gray leaf spot is endemic, have been documented (Latterell and Rossi, 1983; Ward *et al.*, 1996; Nowell, 1997; Ward *et al.*, 1997c). The increase in the incidence and severity of the disease is reported to be associated with the increase in minimum tillage farming practices (Roane *et al.*, 1974; Hilty *et al.*, 1979; Rupe *et al.*, 1982; Payne *et al.*, 1987).

The disease has caused crop losses in most of Central and Southern Africa except Angola, Botswana, Lesotho, Mozambique and Namibia (Lipps *et al.*, 1998; Nowell, 1997). In South Africa, the disease was first reported in KwaZulu – Natal in 1988. The disease has since spread to neighbouring provinces. In recent years the disease has become a serious problem in South Africa, Zimbabwe and Zambia (Ward *et al.*, 1997b; Nowell, 1997; Ward *et al.*, 1999).

In Zambia gray leaf spot disease was first reported from Mkushi in Central Province in 1995 (Kaula, personal communication). The disease became serious from 1997 and is

now known to occur widely in all areas with rainfall of more than 800 mm per growing season.

Spore germination was found to take place 24 hours after the plants were exposed to 12 hours of leaf wetness at 22 – 30 °C (Beckman and Payne, 1982; Stack, 1999). Disease development requires periods of sustained high humidity in order to accommodate the extended periods of growth of *Cercospora zea-maydis* on the leaf surface before penetration of the leaf could occur. This is possible in the field due to a microclimate created by the plant canopy.

Rupe *et al.*, (1982) worked on the influence of the environment and plant maturity on the growth of gray leaf spot disease of maize. They showed that daily long periods of leaf wetness and high relative humidity influenced the incidence of gray leaf spot disease on the crop. These conditions are known to promote conidial germination and germ tube growth of the fungus. Their investigation also revealed that plant maturity played an important role in the initial appearance of the disease symptoms and that the delayed appearance of the initial inoculum did not seem to influence disease development.

Plant maturity is an important factor in the development of gray leaf spot disease in maize late in the growing season (Beckman *et al.*, 1981). High temperature and low rainfall are not limiting factors for gray leaf spot disease development. Severe disease development has occurred after the development of the full plant canopy and is attributed

to the microclimate factors such as relative humidity that are associated with the full plant canopy (Beckman *et al.*, 1981; Rupe *et al.*, 1982).

Beckman and Payne (1983) investigated the cultural techniques and conditions influencing growth and sporulation of *Cercospora zea-maydis*. Using different light regimes, they revealed that continuous light inhibited spore germination, germ tube growth and sporulation. Sporulation was high when the conidia were exposed to 12 hours of light followed by 12 hours of darkness for 14 days in V-8 juice agar. The disease developed optimally at 22-28 °C and relative humidity of more than 90%. However, problems have been experienced in developing the disease on the host under green house conditions (Beckman and Payne, 1983; Latterell and Rossi, 1983).

Payne and Waldron (1983) demonstrated that the presence of plant debris infected with gray leaf spot on the ground increased the severity of the disease. They also showed that *Cercospora zea-maydis* survived as mycelia in the plant debris. These workers also revealed that the amount of inoculum was greatly reduced if the debris was buried 15 cm below the soil surface. From their work it can be assumed that winter ploughing may help to reduce the amount of inoculum levels in a given area and help to minimise disease incidence.

The effect of soil surface residue levels on the progress of gray leaf spot disease in maize was investigated in Ohio, USA during two growing seasons (de Nazareno *et al.*, 1993). They demonstrated that any tillage method that left plant residue on the soil surface

favoured gray leaf spot disease development on maize and that the disease increased proportionately to the amount of plant debris on the soil surface.

Payne *et al.*, (1987) compared the effect of four tillage treatments, namely, fall plough - spring disk, fall disk- spring plough - spring disk, spring plough - spring disk, and no-till, on the development of gray leaf spot disease and the associated numbers of air borne conidia and showed that the levels of air borne conidia were high in no-till plots. The lesions appeared earlier and the disease was greater at each evaluation date in no-till plots than in the other plots. The total yield and yield per plant was much less in no-till plots. A direct relationship between no tillage farming practice and continuous maize production on the incidence and severity of gray leaf spot disease of maize has been shown (Roane, 1950; Hawk *et al.*, 1985).

Bair and Ayers (1986) studied the variability of *Cercospora zeae-maydis* isolates from several geographical regions of eastern United States. They examined the lesion length and disease efficiency in four susceptible maize hybrids and showed that variation existed between isolates obtained from different regions of eastern United States. They suggested that cultivars developed to resist the disease severity should be screened against a wide range of isolates.

Gwinn *et al.*, (1987) investigated the effect of maize plant age and cultivar's resistance to *Cercospora zeae-maydis* and its sensitivity to cercosporin, a phytotoxin produced by *Cercospora* species. Using three cultivars they were able to show that the fungus

penetrated more stomata of older plant tissue if cercosporin was added to the diseased plant tissue. Cercosporin causes reduced ion leakage from the leaf disks and therefore increase penetration by the fungus. From this it became apparent that a real age-dependent resistance to *Cercospora zae-maydis* exists in maize and it could be due to reduced ion leakage in older plants.

2.1 Symptoms of gray leaf spot

Gray leaf spot is a foliar disease and the causal pathogen attacks leaves and sheaths of maize plants. The disease appears as minute spots surrounded by yellow haloes (Shurtleff, 1973; McGee, 1988; Agrios, 1997). The spots enlarge and elongate as streaks before developing their dark grayish rectangular shape, which may coalesce, killing the plant (Latterell and Rossi, 1983). The lesions are gray to tan in colour and are delimited by major leaf veins. Mature lesions are 20-60 mm long and 2-4 mm wide. The pathogen appears towards the anthesis stage of the crop and the attack begins from the lower leaves. It then spreads steadily to the upper leaves.

2.2 Morphology and Physiology

Conidiophores of *Cercospora zae-maydis* emerge in clusters from stromatic tissue in the substomatal chambers. The conidiophores are dark brown in colour and geniculate and bear conidia sympodially (Chupp, 1953; Kingsland, 1963). The conidia are hyaline and measure 30 - 180 μm in length and 4 - 9 μm in width and have 3 to 10 septa (Kingsland,

1963; Latterell and Rossi, 1983; Nowell, 1997). The size and shape of conidia and conidiophores are variable and depend on the substrate on which the pathogen grows and agro-ecological regions they inhabit (Latterell and Rossi, 1983).

2.3 Epidemiology

Cercospora zae-maydis has been found to survive as mycelium in infected plant debris for one year (Payne and Waldron, 1983; Latterell and Rossi, 1983; Ward *et al.*, 1999). It is capable of causing disease when maize is planted the following growing season. The disease appears when the crop has reached the anthesis stage. This is due to the effect of plant canopy, which provides prolonged favourable conditions for the development of the disease. The disease is prevalent in places where minimum tillage is practised (Payne and Waldron, 1983). It is also favoured by relative humidity of about 90% for 12 to 13 hours per day and leaf wetness of 11 to 13 hours per day (Rupe *et al.*, 1982). The environmental conditions are very important in determining the amount of injury that will occur to the crop by the time it reaches maturity (Ward *et al.*, 1999).

2.4 Control of gray leaf spot

Cercospora zae-maydis only attacks maize among the food crops. This means that crop rotation of at least one year can help to reduce the incidence of gray leaf spot disease. Proper management of the plant residue can also help to control gray leaf spot disease. Using disease resistant maize varieties can effectively control gray leaf spot disease of

maize. In the absence of resistant varieties, gray leaf spot tolerant maize varieties can be used to manage the disease. The disease can also be controlled using fungicides.

Gray leaf spot disease of maize can also be controlled using integrated management practices like soil fertility, plant density and irrigation which can influence gray leaf spot disease epidemic (Ward and Nowell, 1998). This is because a soil with high nutritional value will help to increase the tolerance of the plant to the disease. A high plant density facilitates the easy spread of conidia from one plant to the other since it reduces the distance the conidia have to travel before reaching its host. Continuous irrigation increases the period of leaf wetness, which facilitates the development of gray leaf spot disease.

Rainfall and sporulation during early infection cycles have a significant effect on the development of gray leaf spot disease (Ringer and Grybauskas, 1995). Therefore management strategies aimed at decreasing the levels of inoculum during early infection cycles can be effective means of reducing the severity of gray leaf spot disease.

The decision to incorporate a fungicide application in the management of gray leaf spot disease depends on the potential for disease development, the tolerance of the hybrid to the disease and the economic benefit from a fungicide application (Stack, 1999). Fungicide control of gray leaf spot disease is effective if spraying is started after the disease severity reaches 2-3% of leaf area and when lesions are restricted to the basal five leaves of the maize plant (Ward *et al.*, 1997a and 1997b). The frequency and number of

fungicide applications varies with the stage of host development from the time the disease was first apparent. Ward *et al.*, 1997b, showed that the response of the yield to fungicide application is a function of the growth stage of the host when sprays were initiated. It also depends on the amount of disease at spray date, the length of fungicide control and the effective control of the disease by the fungicide through to physiological maturity.

Ward *et al.*, (1996) showed that disease control by fungicides led to high yields in susceptible hybrids than in tolerant hybrids. They also revealed that less susceptible hybrids are likely to require fewer fungicide treatments than more susceptible hybrids and are at a lesser risk of serious yield losses. Thus the effectiveness of a fungicide application is influenced by inoculum pressure present at the time of spraying (Nowell, 1997).

Early Impact is a broad-spectrum systemic and contact fungicide produced by Zeneca Agrochemicals, United Kingdom. It is used for the control of foliar diseases of maize, wheat and barley. Early Impact is marketed as a suspension concentrate. The recommended dosage for Early Impact is 1.25 L in 40 L of water per hectare for aerial application and 1.25 L in 200 – 300 L of water per hectare for ground application. Early Impact is able to offer protection to the plant up to 3 – 4 weeks after application. The compatibility of Early Impact can be adversely affected by the quality of the water used to spray. A trial mixture must be done using water intended for spraying to assess its compatibility (Zeneca Agrochemicals, Fernhurst, Haslemere, Surrey GU27 3JE, UK).

According to the manufacturer, Early Impact fungicide penetrates and moves upwards in the xylem to give full protection. Its mechanism of action is by interfering with the production of egosterol, a vital component of the cell membrane of the pathogen. Early impact application results in fungal cell wall collapse and inhibition of hyphal growth (Zeneca Agrochemicals, UK).

3.0 MATERIALS AND METHODS

3.1 Sample collection

Representative samples were collected from all three agro-ecological regions of Zambia (Fig. 4). The samples were collected from farms of peasant farmers in Livingstone (Region I), Choma and Kabwe (Region II) and Ndola (Region III). The samples were collected from plots within the radius of 2 kilometres of each town (Table 1). The maize was grown under natural weather conditions. The samples were collected when the crop was at the grain filling stage. The disease was allowed to develop naturally under the climatic conditions prevailing in that location. Samples of diseased leaves were collected from each of the sample sites and were replicated three times for each site. After collecting, the samples were put in unused polythene bags in a cool box and taken to the plant pathology laboratory at the University of Zambia for examination. These samples were kept at 8 °C in a refrigerator before being worked on.

Table 1: Agro-ecological regions and number of fields sampled per site (2 km radius range).

REGION	SAMPLE SITE	NO. OF FIELDS
I	Livingstone	4
II	Choma	5
II	Kabwe	8
III	Ndola	6

3.2 Isolation and observation

Sections of diseased leaf tissues (30 x 5 mm in size) were cut from each of the infected leaves and sterilised in 2% sodium hypochlorite solution for 5 minutes and then rinsed three times in sterile distilled water. After this, the samples were placed on sterile moist blotters in a sterile petri dish. Ten sections of diseased tissue were placed in each petri dish, and ten petri dishes were prepared for each field. The petri dishes were then left at 25 °C for 5 days to allow the pathogen to develop and sporulate.

The sporulating diseased sections were examined for the presence of conidia. Those containing the conidia were suspended in 10 cm³ of sterile distilled water and vigorously shaken to dislodge the conidia. A drop of the conidial suspension was then put on a microscope slide and examined at magnification of x10 objective with a x10 eyepiece. Measurements of length and width of the conidia and counts of numbers of septa in each were made and recorded. One hundred measurements of conidia were taken from each sample.

3.3 Effectiveness of Early Impact fungicide

A maize variety known to be susceptible to gray leaf spot disease was used to assess the effectiveness of Early Impact fungicide. This is because the effect of a fungicide treatment on a susceptible variety is more evident than on a tolerant one. SC 401, a maize

variety rated 9 according to the 1 to 9 gray leaf spot disease rating of Seed Co. Ltd. varieties done in Zimbabwe, was used in this investigation.

3.3.1 Experimental set-up

A Completely Randomised Design was used in the set up of this experiment. The experiment included four treatments replicated three times. The treatments included a control, a single fungicide application, two fungicide applications and three fungicide applications during the growing season. The experiment was conducted at two sites, the University of Zambia (Unza) School of Agriculture field station and Mt. Makulu Research station in Chilanga. Unza was selected because of its easy access by the researcher and Mt. Makulu was selected due to its being a rainfall catchment area (Appendix II). Both plots had maize in them the previous season and the maize debris was not removed but was ploughed in before planting.

The seeds were planted in rows of 10 m long and 0.75 m apart at a seed rate of 20 kg ha⁻¹ and a 2 m distance separated the plots. Each plot consisted of 10 rows and was exposed to normal agronomic procedures. The basal fertiliser (D-Compound) was applied at planting time at a rate of 200 kg ha⁻¹ while top dressing (Urea) was applied at a rate of 150 kg ha⁻¹ when the crop was a month old from the day of emergence. The crop was regularly surveyed for incidence of gray leaf spot and other foliar diseases.

3.3.2 Disease rating

Disease rating was done three times at three-weeks interval during the growing period of the crop for all the plots and this was after fungicide application had commenced. It was done when the crop was 72, 94 and 113 days after emergence at Unza, and 78, 100 and 113 days after emergence at Mt. Makulu. At each rating, 20 plants were assessed for disease incidence according to the Food and Agricultural Organisation (FAO) methods of disease assessment (FAO, 1971).

The number of healthy and diseased leaves was recorded under the 0-9 grades from the ear leaf as follows:

0%	leaf area infected = 0 grade
1-10%	leaf area infected = 1 st grade
11-20%	leaf area infected = 2 nd grade
21-30%	leaf area infected = 3 rd grade
31-40%	leaf area infected = 4 th grade
41-50%	leaf area infected = 5 th grade
51-60%	leaf area infected = 6 th grade
61-70%	leaf area infected = 7 th grade
71-80%	leaf area infected = 8 th grade
81-100%	leaf area infected = 9 th grade

The percentage disease index was calculated using the following formula:

$$\text{Percentage disease index} = \frac{\text{Sum of numerical values} \times 100}{\text{Total number of leaves observed} \times \text{maximum grading (9)}}$$

Spraying did not commence until the crop was about 60 days old from emergence. This was the time when disease incidence had reached about 5% (Nowell, 1997; Ward *et al.*, 1997a and 1997b). The first fungicide spray was applied when the crop was 61 days old from emergence at Unza and at 64 days old from emergence at Mt. Makulu. This was followed by another fungicide application 21 days later (at 82 and 85 days) for those plots that required a second fungicide application. A third fungicide application was applied 21 days later (103 and 107 days at Unza and Mt. Makulu respectively). The fungicide was applied at the rate of 1.25 L per hectare in 200 L of water using a Knapsack sprayer (CP3. ICI, Fernhurst Haslemere, Surrey, England), whose delivery rate was 200 L ha⁻¹.

The crop was harvested 156 days after planting for the control plots while the treated plots were harvested two weeks later. This was because the control plots dried much earlier than the treated plots. At the time of harvest the grain moisture content was 20% and the maize was sun dried to 12% moisture content for the control and treated plots. The yield per plot was calculated in kilograms and was then converted to kilograms per hectare. Analysis of variance was used to compare yields while the least significant difference (LSD) was used to separate means.

3.4 Analysis of results

Statistical analysis of the experimental results to test the significance of variations between mean length, width and number of septa of conidia, for the different sites were carried out using Statistix for Windows. (Analytical Software 1996, Tallahassee, Florida). The confidence limit chosen for the analysis was 0.05. This is because a very small probability level (P) would mean that the hypothesis must be accepted as it is (Barley, 1959).

4.0 RESULTS

4.1 Conidial morphology

The results on the morphology of the conidia were analysed by looking at the length and width as well as the number of septa.

4.1.1 Length

The analysis of variance of the length of conidia revealed that there were significant differences between fields of the same site (Appendix XII). The least significant difference for separation of means indicated significant differences between the mean lengths of fields of the same site as seen in Ndola 1 (77.798 μ m) that was significantly different from Ndola 2 (74.712 μ m) and Ndola 4 (83.125 μ m).

The analysis also showed significant differences between the sampled agro-ecological sites. A comparison of means by least significant differences showed that Ndola (Region III), mean length of 78.491 μ m, was significantly different from the rest of the sites while Kabwe (Region II), and Livingstone (Region I), with mean lengths of 75.709 μ m and 75.741 μ m respectively, were not significantly different from each other and Choma (Region II), mean length of 74.353 μ m, stood on its own (Table 2).

Further analysis of variance of length of conidia by regions revealed significant differences between the agro-ecological regions (Appendix XV). A least significant difference (LSD) for separation of means showed that region III was significantly different from the other two regions (Table 2). Regions I and II were not significantly different from each other.

Table 2: Statistical analysis of spore dimension measured in length and width (μm), and number of septa in each as observed by region and sample site.

REGION	SAMPLE SITE	LENGTH	WIDTH	NO. OF SEPTA
I	Livingstone	75.741 μm b	9.914 μm c	7.715 e
II	Choma	74.353 μm b	9.998 μm d	7.705 f
II	Kabwe	75.709 μm b	9.990 μm d	7.966 f
III	Ndola	78.491 μm a	10.000 μm d	8.620 g

*Means followed by the same letter are not significantly different at $P\leq 0.05$ probability level using Least Significant Difference (LSD) for separation of means.

4.1.2 Width

An analysis of variance of the width of conidia by field showed significant differences from one field to the other with an F value of 2.98 (Appendix XVI) at the probability level of $P \leq 0.05$. Some fields in Choma were not significantly different from fields in Ndola while others were not significantly different from those found in Livingstone (Appendix XVII). The same trend was observed for the fields in Kabwe, which were found to occur in three categories. Further analysis of variance of width of conidia by sample site showed significant differences between the different sites. However, Ndola ($10.00\mu\text{m}$), Choma ($9.99\mu\text{m}$) and Kabwe ($9.99\mu\text{m}$) were not significantly different from each other but were significantly different from Livingstone with a mean width of $9.914\mu\text{m}$ (Table 2).

Further analysis of variance of the width of conidia by region revealed significant differences with an F value of 19.65 (Appendix XIX). A mean separation by least significant differences revealed that regions II and III were not significantly different from each other but were significantly different from region I (Table 2).

4.1.3 Number of septa

The analysis of variance of number of septa by field revealed significant differences among the fields of the same sample site (Appendix XX). There were very few fields that were not significantly different from each other. An analysis of variance of number of

septa of conidia by sample site also showed significant differences between the sites with an F ratio of 67.74 and degree of freedom for error of 3 (Appendix XXII). The separation of means by LSD showed that Choma and Kabwe (mean number of septa of 7.705 and 7.966 respectively) were not significantly different from each other (Table 2). Ndola and Livingstone (mean number of septa of 8.62 and 7.71 respectively) were each significantly different from the other sites.

Further analysis of number of septa by region revealed significant differences among the agro-ecological regions with an F value of 94.21 at probability level of $P \leq 0.05$. (Appendix XXIII). Each of the regions was significantly different from the other (Table 2), an indication that the number of septa differs from one region to the other.

4.2 Effectiveness of Early Impact fungicide

The effectiveness of early impact fungicide was analysed in two ways. This was done by looking at the effect of the fungicide on the incidence of gray leaf spot, and its effect on the yield.

4.2.1 Effect of Early Impact fungicide on incidence of gray leaf spot disease

An analysis of variance of the disease incidence by site showed significant differences in the disease index from the two experimental sites. The first disease rating values

following the initial fungicide application from Unza were significantly different from the Mt. Makulu ratings with an F value of 7.50 (Appendix VII). The ratings done after the second fungicide application showed that only the ratings on the untreated plots at both sites were significantly different from the treated ones.

The disease incidence at 113 days after emergence revealed significant differences between the disease ratings of the different treatments (Appendix X). The incidence of disease on the untreated plots was not significantly different from each other, with mean disease index of 68.22 and 66.04 at Mt. Makulu and Unza respectively. However, these were significantly different from those that received one fungicide treatment with disease index of 10.71 and 13.04 at Mt. Makulu and Unza respectively, and from those that received two treatments with disease index of 2.91 and 3.69 at Mt. Makulu and Unza respectively. The disease incidence ratings from those that received two fungicide applications were not significantly different from those that received three (1.73 and 1.49 at Mt. Makulu and Unza respectively) fungicide applications (Appendix XI).

The incidence of gray leaf spot disease increased as the crop aged in the untreated plots. This was seen by the increase in the disease index from 33.58 at onset of disease rating to 66.06 at the end for the plots at Unza and from 43.15 to 68.22 at Mt. Makulu. Generally the disease index at Mt. Makulu was higher than the disease index at Unza.

There was a steady decline in the disease index for the sprayed plots (Fig. 5 and 6). The plots that were sprayed once showed a decline in disease index at first but later the

disease index increased. The plots that received two and three fungicide applications did not show an increase in the disease index but there was continuous reduction in the disease index to about 2.0. This was a very big reduction from the initial disease index of about 30. Similar trends were observed for both Mt. Makulu and Unza. The treated plots looked healthier, greener and dried much later compared to the untreated plots. Figure 7(a) was not treated with the fungicide and therefore looks drier compared to the fungicide treated crop in Figure 7(b), which is greener.

4.2.2 Effect of Early Impact fungicide on yield

Analysis of variance of yield by experimental sites revealed significant differences in the yield for the two experimental sites with an F ratio of 10.21 (Appendix VI) at the probability level $P \leq 0.05$. The total yields from the two sites were significantly different from each other using LSD for separation of means (Table 3). The mean total yields from Unza were lower than the mean total yields from Mt. Makulu in by $1948.4 \text{ kg ha}^{-1}$.

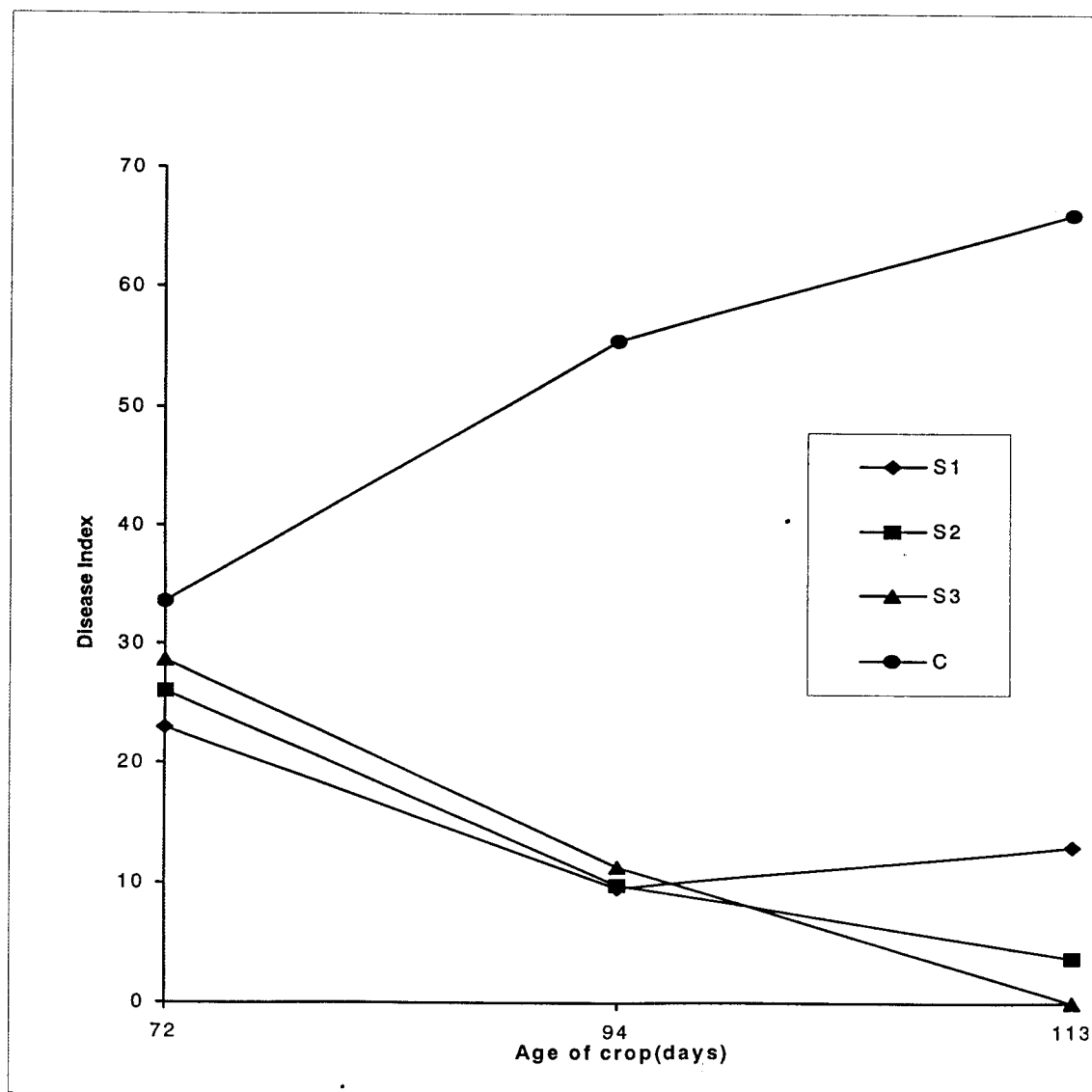


Fig. 5: Disease index of gray leaf spot caused by *Cercospora zae-maydis* on maize variously treated with Early Impact fungicide, at the University of Zambia, School of Agriculture, field station, where S1-one fungicide spray, S2-two fungicide sprays, S3- three fungicide sprays and C - control.

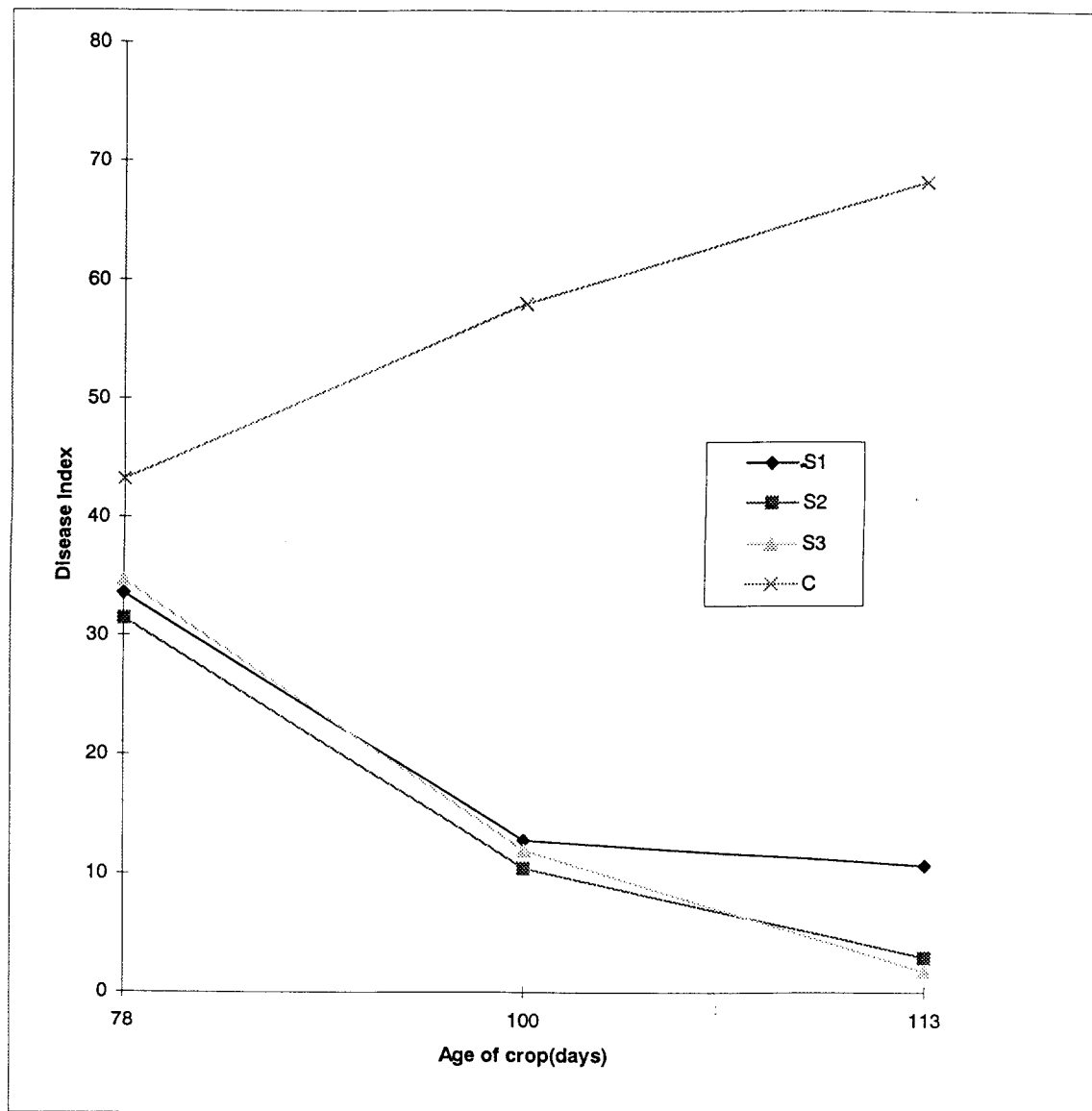
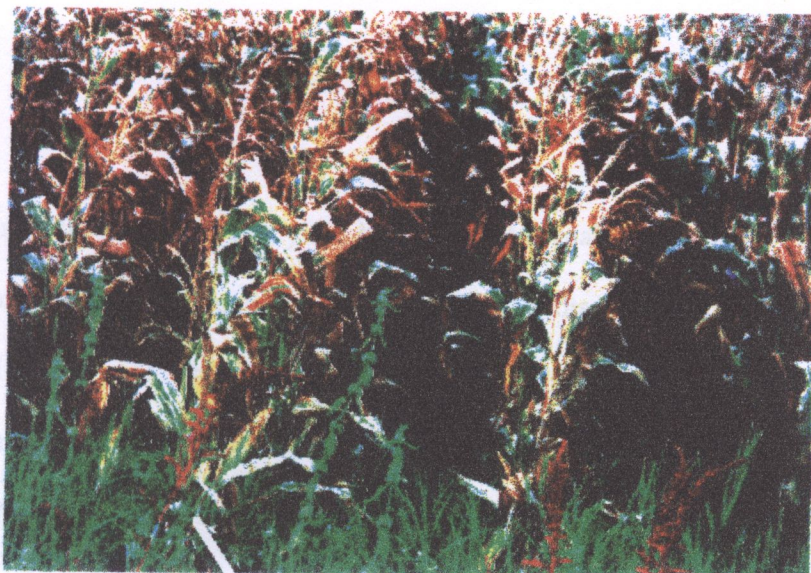


Fig.6: Disease index of gray leaf spot caused by *Cercospora zae-maydis* on maize variously treated with Early Impact fungicide at Mt. Makulu Research Station, Chilanga, where S1- one fungicide spray, S2- two fungicide sprays, S3- three fungicide sprays and C- control.



(a)



(b)

Fig.7: Control of gray leaf spot of maize caused by *Cercospora zeae - maydis* by Early Impact fungicide (a) untreated (b) received two fungicide applications.

Further analysis of variance of yield by treatment revealed significant differences among the treatment means (Appendix V). The untreated plots had the minimum yield and this was significantly different from those that received one fungicide application (Table 4). The treatments that received two and three fungicide applications were not significantly different from each other for both experimental sites.

Minimum yield was obtained at Unza ($3745.5 \text{ kg ha}^{-1}$) for the control as compared to the control from Mt. Makulu that had a yield of $5509.0 \text{ kg ha}^{-1}$ (Table 4). The yield differences for Unza, between the control and the single and double fungicide applications was significant, ranging from 3700 kg ha^{-1} for the control and from 5600 to 7100 kg ha^{-1} for the treated areas. The yield differences for Mt. Makulu, between the control and the single and double fungicide applications, although insignificant, were noticeable. It varied between 5500 kg ha^{-1} for the control and from 7800 to 8700 kg ha^{-1} for the sprayed areas. 3000 kg ha^{-1} difference can feed about 6000 people at 500g per person per day (FAO, 1997).

Table 3: Means of total yield (kg ha⁻¹) of maize by experimental site.

SITE	MEAN (x 1000 kg ha⁻¹)	GROUP STD DEVIATION
Mt. Makulu	7.4839a*	1.5193
Unza	5.355b	1.4669
AVERAGE	6.5097	1.4933

*Means followed by the same letters are not significantly different at $P \leq 0.05$ probability level using LSD for separation of means.

Table 4: Means of analysis of yield of maize by treatment at MT Makulu (MtM) and University of Zambia (Unza).

TREATMENT	MEAN (x 1000 kg ha⁻¹)
Unsprayed-Unza	3.7455d*
Once sprayed-Unza	5.6190c
Twice sprayed-Unza	5.5781c
Thrice sprayed- Unza	7.1994b
Unsprayed-MtM	5.5090c
Once sprayed-MtM	7.8974a
Twice sprayed-MtM	7.8053a
Thrice sprayed-MtM	8.7238a

*Means followed by the same letter are not significantly different at $P \leq 0.05$, probability level using LSD for separation of means.

4.3 Economic benefits of Early Impact

A statistical analysis was done to determine the benefits of applying Early Impact fungicide on maize infected with gray leaf spot disease. The analysis indicated which level of fungicide application would benefit the farmers. Adding all the expenses that a farmer would have incurred in order to be able to grow the crop did the analysis. The gross field benefits of the experimental plots were calculated from the adjusted yield of the actual yield (CIMMYT, 1988). Then the expected net benefits from the crop were calculated. Table 5 shows the yield from the different treatments at the two experimental sites.

The costs that vary were calculated and were included in the determination of the economic benefits of applying Early Impact fungicide. The total costs that vary show the differences in costs between the different treatments of the experiment. A partial budget for the fungicide experiment indicating the total costs that vary and the net benefits of applying Early Impact fungicide are outlined in Table 6.

The dominance analysis revealed that a single fungicide application (Table 7) dominated in the net benefits of the yield than the one with two fungicide applications. This meant that the treatment with two fungicide applications could not be used in this analysis because its net benefits were lower than the net benefits for the treatment with a single fungicide application.

Table 5: Mean maize grain yield (kg ha⁻¹) for the treatments at Mt. Makulu and Unza.

Site	TREATMENT			
	No fungicide spray	One spray	Two sprays	Three sprays
	Average yield			
Unza	3745.5	5619.0	5578.1	7199.4
Mt. Makulu	5509.0	7897.4	7805.3	8727.7
Average yield	4627.25	6758.2	6691.7	7963.55

Table 6: Net benefits (Zambian Kwacha) of applying Early Impact fungicide on maize to control gray leaf spot caused by *Cercospora zae-maydis*.

	TREATMENT			
	No spray	Single spray	Two sprays	Three sprays
Average yield (kg ha ⁻¹)	4627.25	6758.2	6691.7	7963.55
Adjusted yield (kg ha ⁻¹)	933.2	5744.5	5687.9	6769.0
Gross field benefits (k ha ⁻¹)	1,376,620.00	2,010,575.00	1,990,765.00	2,369,150.00
Cost of fungicide (1.25L ha ⁻¹)	—	68,750.00	132,500.00	196,250.00
Labour to apply fungicide	—	20,000.00	40,000.00	60,000.00
Hiring sprayer (in K)		32,000.00	64,000.00	96,000.00
Total costs that vary (k ha ⁻¹)	—	120,750.00	236,500.00	352,250.00
Net Benefits (K ha⁻¹)	1,011,620	1,524,825.00	1,389,265.00	1,651,900.00

The dominance analysis revealed that the treatment with a single fungicide application (Table 7) dominated the net benefits of the yield from the treatment with two fungicide applications. This meant that the one with two fungicide applications could not be used in this analysis because its net benefits were lower than the net benefits for the treatment with a single fungicide application.

4.3.2 Net Benefit curve

The net benefit curve enables one to tell which level of fungicide application is beneficial to the farmer by simply looking at the graph. It saves the time of calculating the benefit if one is quickly going through the document. This is where the net benefits are plotted against the total costs that vary for the given treatment. The net benefit curve for Early Impact fungicide indicated that a single fungicide application could benefit the commercial farmer (Fig. 8). Two fungicide applications showed that it gave a net benefit lower than the net benefit for the single application and therefore cannot be considered appropriate in the analysis and is dominated.

Table 7: Dominance analysis for Early Impact fungicide for the control of gray leaf spot on maize caused by *Cercospora zae-maydis*.

FUNGICIDE TREATMENT (NO. OF SPRAYS)	TOTAL COSTS THAT VARY (K/ha)	NET BENEFITS (K/ha)
None	None	1,011,620.00
One	120,750.00	1,524,825.00
Two	236,500.00	1,389,265.00 D
Three	352,250.00	1,651,900.00

D is a dominated treatment.

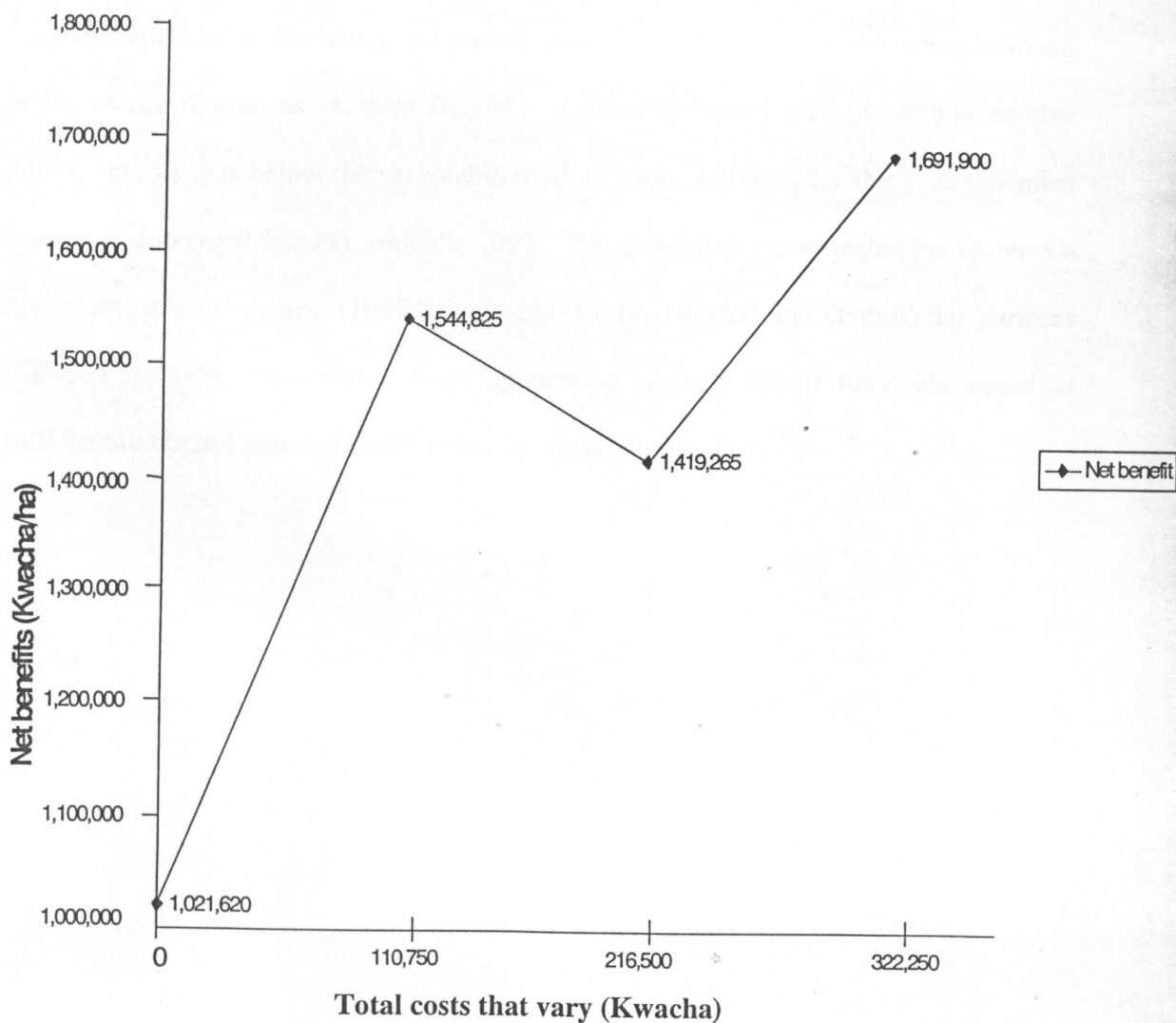


Fig. 7: Net benefit curve for the control of gray leaf spot caused by *Cercospora zeae-maydis* by Early Impact fungicide.

The plotting of a net benefit curve showed that a single application of Early Impact fungicide would give the farmer a marginal rate of return of 425% (Table 8). The single fungicide application dominated the second application and therefore was not considered in the marginal analysis. A third fungicide application would give the farmer another 54.9% but this was below the acceptable minimum rate of return for this recommended domain (commercial farmer), which is 100%. This is because a new technique requires a maximum rate of return (100%) if it has to be adopted by commercial farmers (CIMMYT, 1988). Therefore a single application of Early Impact fungicide would be sufficient to control gray leaf spot disease on maize.

Table 8: Marginal rate of return (Zambian Kwacha ha⁻¹) of Early Impact on maize infected with gray leaf spot caused by *Cercospora zeae-maydis*.

Treatment	Costs that vary	Marginal costs	Net benefits	Marginal net benefits	Marginal rate of return (%)
1	0.00	120,750.00	1,011,620.00	513,205.00	425.0
2	120,750.00		1,524,825.00		
3	352,250.00	231,500.00	1,651,900.00	127,075.00	54.9

5.0 DISCUSSION

The results of this study relied on natural infection for the development of the gray leaf spot disease on maize. This meant that the results were reliable because;

- (i) the disease developed from naturally occurring inoculum which represented the range of strains of *Cercospora zae-maydis* present in the given field and site.
- (ii) the infections occurred continuously over a period of time, without being affected by artificial dosage administration, and
- (iii) that the strains were naturally aggressive, as they were not maintained in culture or selected for exceptional virulence.

5.1 Conidial morphology

There was significant variation in the length of conidia of *Cercospora zae-maydis* from those of regions I and II as compared to region III. This could have been due to the differences in the total amount of rainfall received by region III (Appendix I). Ndola received the maximum amount of rainfall of 769.5 mm from October 1999 to March 2000. Within the same period, Choma and Kabwe received 597.5 mm and 751.7 mm respectively while Livingstone received 623.7 mm of rainfall. The width of conidia showed that there were no significant differences between agro-ecological Regions II and III but these were significantly different from Region I. It has been proved that high

rainfall favours the development of gray leaf spot disease of maize (Bair and Ayers, 1986), which has an effect on the morphology.

Similarly, the presence of variability within and between agro-ecological regions was observed in the number of septa of conidia. This again could be due to the differences in climatic conditions within and between the regions. This is because fields also showed wide variation in the length and width of conidia as well as in the number of septa. The conidial morphology has been studied by several workers who reported that conidial length and width can vary among different isolates (Bair and Ayers, 1986; Donahue *et al.*, 1991). Similar differences in conidial length and width were also found in this study.

The number of septa of conidia was found to be significantly different from one agro-ecological region to the other. This could be due to the differences in the amount of rainfall, relative humidity as well as the minimum and maximum temperatures (Appendices I, III and IV) each region experienced within the growing season. The effect of environmental conditions on the development of gray leaf spot disease on maize has been shown by several workers (Beckman and Payne, 1982; Latterell and Rossi, 1983; Ward *et al.*, 1996; Nowell, 1997; Ward and Nowell, 1998). This research has shown that differences in the climatic conditions can bring about variations in the morphology of *Cercospora zea-maydis*. It also implies that farmers in areas that receive higher rainfall per year need to grow maize varieties resistant to gray leaf spot disease because of the expected higher incidence of the disease. Conidial size can be an indication of the

presence of pathotypes within the species. Whether this is so is something that needs further investigation.

5.2 Effectiveness of Early Impact fungicide

The absence of significant differences between fungicide treatments in the disease index at the time of the first disease rating could mean that the trend of disease control of Early Impact fungicide was the same. At the time of the second disease rating there were no significant differences among the treated plots. This could imply that after the second fungicide application the disease levels were the same for all the treated plots. However, differences among treatments were observed after the third fungicide application. Plots that received a single fungicide application were significantly different from the rest with disease indexes of 12.77 and 9.53 for Mt. Makulu and Unza respectively compared to those that received two and three fungicide sprays with disease indexes of 10.45 and 9.82 and 12.06 and 11.34 at Mt. Makulu and Unza respectively.

The disease index in the untreated plots increased with time indicating that there was continuous re-infection of the crop by the pathogen. The secondary inoculum could have come from diseased plant tissue that continuously developed conidia and caused fresh infections (Beckman and Payne, 1982; Latterell and Rossi, 1883; Stromberg, 1986; Ward *et al.*, 1999). In the plots with a single fungicide application, the disease incidence decreased initially and then increased later. This could be attributed to the fungicide that was only able to control gray leaf spot disease for a short period of time. After a period of

four to five weeks, the fungicide lost its potency and therefore could no longer offer further protection leading to the crop. Perhaps it could also have been due to inadequate fungicide spread on the host's surface to give enhanced protection.

The disease index in the plots that received two fungicide applications did not increase, an indication that Early Impact fungicide was able to effectively control gray leaf spot disease throughout the growth period. Similar observations have been made by Ward *et al.*, 1997c. The plots that received three fungicide applications did not show an increase in the disease index, indicating that the disease was effectively controlled. This means that a single fungicide application was necessary to control gray leaf spot disease on maize if the disease pressure is low. Two fungicide applications would be necessary if the disease pressure is high and if it disease appears early in the development of the crop. Any additional fungicide application would be a waste of chemical, time and money, as it would have no impact on the grain yield.

5.3 Effect of Early Impact fungicide on yield

There were significant differences in the yield of the plots treated with the fungicide and the untreated ones. It varied from 5619.0 to 8723.8 kg ha⁻¹ for the treated plots while the control was about 3700 kg ha⁻¹. The absence of significant differences between plots that received one and two fungicide applications show that only one fungicide application is necessary to control gray leaf spot disease. The effective control of gray leaf spot disease

of maize occurs especially if the disease appears late in the developmental stages of the crop (Ward *et al.*, 1996; Ward, *et al.*, 1997a and 1997b; Ward and Nowell, 1998).

The yield from the plots sprayed three times was significantly different from the rest of the treatments for Unza. This indicates that the three-sprayed plots were not infected during their development. However, for Mt. Makulu, those that received three fungicide sprays were not significantly different from those that received two fungicide sprays. This was because the treated crop remained green for a longer period when compared to the untreated ones. The grain filling period was therefore much longer in the treated plots and therefore gave a higher yield (Ward, *et al.*, 1997b).

The significant differences observed between the two experimental sites could have been due to the differences in the climatic conditions at the two locations (Appendix II). The amount of rainfall received by the two experimental sites was different just as were the minimum and maximum temperatures. High rainfall coupled with high average relative humidity favours the development of gray leaf spot disease on maize (Donahue *et al.*, 1991). The maximum temperatures were generally higher at Mt. Makulu than at Unza (Appendix II). The weather conditions for Unza are those indicated under Lusaka in the appendix. This could explain why gray leaf spot disease appeared earlier at Mt. Makulu.

The lower yields at Unza compared to Mt. Makulu yields could have been due to the differences in the nutritional value of the soil (Donahue *et al.*, 1991). It could also have been due to the presence of other pathogens like maize rust (*Puccinia sorghi*) and leaf

spots (*Helminthosporium* species) that were observed earlier at the Unza plots. This is because an increase in the number of pathogens attacking a crop reduces the leaf area available for photosynthesis leading to the decrease in the yield (Ward *et al.*, 1997b). Stalk rot was also observed on the crop, especially in the controls, since maize gray leaf spot disease predisposes plants to other pathogens. It could also have been due to high disease pressure at Mt. Makulu than at Unza, which had a relatively lower disease pressure. This is because high disease pressure responds more to fungicide application (Nowell, 1997; Ward *et al.*, 1997c; Ward and Nowell, 1998).

5.4 Economic benefits of Early Impact fungicide

This study has shown that the net benefits from a control is about K1, 000 000.00 while the single and double fungicide sprays gave about K1, 500 00.00 and K1, 389 000.00 net benefits respectively. A single fungicide spray gave more net benefits than those that received two fungicide sprays indicating that a single fungicide spray is able to effectively control gray leaf spot.

A marginal rate of return analysis has shown that a single application of Early Impact fungicide on maize infected with gray leaf spot was able to give the farmers a marginal rate of return of 425%. This large figure is usually beneficial to the farmer especially in the trial period of using a new fungicide. This is achievable if the application of the fungicide was done before disease pressure reached 3-5% and if it appeared late during the growth of the crop (Ward *et al.*, 1997c). Two fungicide applications could only be

necessary to control gray leaf spot disease if the disease pressure was high and if the disease appeared early during the life cycle of the crop. Fungicide application is only beneficial to commercial farmers and not to small-scale farmers. This is because the fungicide is costly to the small-scale farmers considering the cost of the fungicide and the small crop acreage grown (Ward *et al.*, 1999).

6.0 CONCLUSION

This study has shown that there is variability in the morphology of conidia of *Cercospora zae-maydis*. This is evident in the length and width of conidia as well as in the number of septa possessed by each conidium that vary within and between agro-ecological regions of Zambia. This variation is due to differences existing in the climatic conditions in the agro-ecological regions. Whether this variation is due to the presence of pathotypes in these growing areas, is something that needs further investigation.

This study has also demonstrated that a single application of Early Impact fungicide is able to effectively control gray leaf spot disease on maize. A single fungicide spray of Early Impact will effectively control gray leaf spot if the disease appear late in the growth stage of the crop and if the disease pressure is low. Two fungicide sprays are only necessary if the disease pressure is low and usually, is not beneficial to the farmer. In such cases the farmers are advised to use other disease management strategies.

Early Impact fungicide is able to increase the maize grain yield when applied to a crop giving an increase of about 3000 kg ha^{-1} when compared to the control. The yields from the plots that received one and two fungicide sprays were not very significant when analysed, meaning that a second spray does not have a big effect on the yield. A single fungicide application of Early Impact gives an economical net benefit of about 425%. An additional fungicide spray will only give the farmer a marginal rate of return of about 54%. This figure is too low especially when the high cost of the fungicide and the small

crop acreage cultivated is considered. The commercial farmers who can afford the high cost of fungicide can appreciate the use of fungicides. In case of peasant farmers, it is recommended that they use other management strategies like the use of resistant or tolerant varieties where available.

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APPENDIX I: Rainfall totals (mm) for 1999/2000 growing season for the sample sites in the three Agro-ecological regions of Zambia.

STATION	YEAR	MONTH	VALUE
CHOMA	1999	October	3.4
		November	72.2
		December	78.6
	2000	January	175.8
		February	267.5
		March	-99.0*
		April	47.4
		May	59.4
KABWE	1999	October	0.0
		November	26.6
		December	125.9
	2000	January	196.8
		February	207.9
		March	194.5
		April	0.0
		May	8.4
LIVINGSTONE	1999	October	12.4
		November	22.5
		December	77.2
	2000	January	136.8
		February	290.9
		March	83.9
		April	17.5
		May	5.5
NDOLA	1999	October	13.1
		November	147.1
		December	86.6
	2000	January	181.9
		February	190.2
		March	150.2
		April	5.7
		May	3.0

*-99.0 refers to missing data.

Source: Zambia Meteorological Department, Lusaka.

APPENDIX II: Rainfall totals (mm), Relative Humidity and Minimum and maximum temperatures (°C) for experimental sites at Lusaka and Mt. Makulu.

STATION	YEAR	MONTH	MINIMUM	MAXIMUM	RELATIVE HUMIDITY	RAINFALL
LUSAKA	1999	October	17.1	29.7	55.9	12.5
		November	17.4	30.2	67.0	36.9
		December	17.2	28.6	-99.0*	91.3
	2000	January	17.9	27.0	-99.0*	281.7
		February	-99.0*	-99.0*	94.6	308.1
		March	17.5	26.6	93.1	225.0
		April	15.9	26.0	87.5	7.6
MT. MAKULU	1999	May	-99.0*	-99.0*	82	7.0
		October	17.5	30.7	8.0	9.9
		November	18.3	30.9	55.3	41.8
	2000	December	17.9	28.9	67.9	196.3
		January	17.9	27.8	76.7	235.0
		February	17.8	27.3	80.9	122.8
		March	17.8	27.5	76.3	179.2
		April	15.7	26.7	72.0	18.0
		May	-99.0*	-99.0*	65.0	-99.0*

*-99.0 shows missing data.

Source: Zambia Meteorological Department, Lusaka.

**APPENDIX III: Monthly minimum and maximum temperatures (°C) for 1999/2000
growing season for the sample sites in the three Agro-ecological
regions of Zambia.**

STATION	YEAR	MONTH	MINIMUM TEMPERATURE	MAXIMUM TEMPERATURE
CHOMA	1999	October	15.2	29.9
		November	16.3	30.6
		December	16.8	27.9
	2000	January	17.3	26.9
		February	17.2	26.4
		March	-99.0*	-99.0*
		April	13.4	25.8
		May	10.7	23.7
KABWE	1999	October	16.7	30.5
		November	18.1	30.9
		December	18.2	29.6
	2000	January	18.4	27.5
		February	17.9	27.0
		March	18.0	27.3
		April	20.4	26.2
		May	18.3	25.8
LIVINGSTONE	1999	October	18.5	33.6
		November	17.4	33.8
		December	18.6	32.0
	2000	January	18.2	29.2
		February	17.7	28.3
		March	17.6	29.3
		April	13.6	29.8
		May	11.6	27.3
NDOLA	1999	October	15.7	31.5
		November	17.2	30.2
		December	17.0	29.9
	2000	January	17.6	28.3
		February	17.9	27.1
		March	17.3	27.4
		April	14.8	28.9
		May	11.5	28.3

*-99.0 is equal to missing data.

Source: Zambia Meteorological Department, Lusaka.

APPENDIX IV: Monthly Relative humidity for the 1999/2000 growing season for the sample sites in the three Agro-ecological regions of Zambia.

STATION	YEAR	MONTH	RELATIVE HUMIDITY
CHOMA	1999	October	45.8
		November	51.3
		December	68.5
	2000	January	79.4
		February	75
		March	77
		April	72
		May	75
KABWE	1999	October	49.1
		November	56.3
		December	65.2
	2000	January	80.2
		February	83.4
		March	85.1
		April	78.1
		May	59.0
LIVINGSTONE	1999	October	37.9
		November	48.1
		December	63.8
	2000	January	73.8
		February	78.5
		March	77.6
		April	66.2
		May	-99.0*
NDOLA	1999	October	46.7
		November	64.5
		December	63.5
	2000	January	73.8
		February	76.6
		March	76.2
		April	61.4
		May	55.3

*-99.0 is equal to missing data.

Source: Zambia Meteorological Department, Lusaka.

APPENDIX V: Analysis of variance of yield by treatment.

SOURCE	DEGREE OF FREEDOM	SUM OF SQUARE	MEAN SQUARE	F value	P
Between	7	57.8556	8.26508	9.46	0.0001
<i>Within</i>	<i>16</i>	<i>13.9827</i>	<i>0.87392</i>		
Total	23	71.8382			

APPENDIX VI: Analysis of variance of yield by experimental site.

SOURCE	DEGREE OF FREEDOM	SUM OF SQUARE	MEAN SQUARE	F value	P
Between	1	22.7766	22.7766	10.21	0.0042
Within	22	49.0617	2.23007		
Total	23	71.8382			

APPENDIX VII: Analysis of variance of disease index at 72 days after emergence of maize crop.

SOURCE	DEGREE OF FREEDOM	SUM OF SQUARE	MEAN SQUARE	F value	P
Between	1	374.302	374.302	7.50	0.0120
Within	22	1097.37	49.8805		
Total	23	1471.67			

APPENDIX VIII: Means of disease index of *Cercospora zae-maydis* on maize at 96 days after emergence for both experimental sites at Mt. Makulu (MtM) and University of Zambia (Unza).

TREATMENT	MEAN	GROUP STD DEVIATION
Unsprayed-MtM	57.963a*	5.8797
Once sprayed-MtM	12.778b	7.6289
Twice sprayed-MtM	10.448b	1.8192
Thrice sprayed-MtM	12.060b	1.1865
Unsprayed-Unza	55.456a	4.0614
Once sprayed-Unza	9.5343b	0.6877
Twice sprayed-Unza	9.8207b	2.6696
Thrice sprayed-Unza	11.347b	0.7823
TOTAL	22.426	3.9083

*Means followed by the same letters are not significantly different from each other at $P \leq 0.05$, probability level using LSD for separation of means.

APPENDIX IX: Analysis of variance of disease index at 100 days after emergence.

SOURCE	DEGREE OF FREEDOM	SUM OF SQUARE	MEAN SQUARE	F value	P
Between	7	9437.11	1348.16	88.26	0.0000
Within	16	244.392	15.2745		
Total	23	9681.51			

APPENDIX X: Analysis of variance of disease index at 113 days after emergence.

SOURCE	DEGREE OF FREEDOM	SUM OF SQUARE	MEAN SQUARE	F value	P
Between	7	17416.7	2488.10	271.41	0.0000
Within	16	146.680	9.16748		
Total	23	17563.4			

APPENDIX XI: Means of disease index of *Cercospora zea-maydis* on maize, at 113 days after emergence, for both experimental sites at Mt Makulu (MtM) and University of Zambia (Unza).

TREATMENT	MEAN	GROUP STD DEVIATION
Unsprayed-MtM	68.223a*	2.1158
Once sprayed-MtM	10.718b	2.7441
Twice sprays-MtM	2.9123c	1.5387
Thrice sprays-MtM	1.7373c	0.1692
Unsprayed-Unza	66.040a	3.5670
Once sprayed-Unza	13.049b	6.7464
Twice sprays-Unza	3.6983c	0.7995
Thrice sprays-Unza	1.4947c	0.2460
TOTAL	20.984	3.0278

*Means followed by the same letter are not significantly different at $P \leq 0.05$, probability level using LSD for separation of means.

APPENDIX XII: Analysis of variance of length of conidia by field.

SOURCE	DEGREE OF FREEDOM	SUM OF SQUARE	MEAN SQUARE	F value	P
Between	22	16692.6	758.754	11.35	0.0000
Within	2446	163554	66.8660		
Total	2468	180247			

APPENDIX XIII: Mean length (μm) of conidia of *Cercospora zeae-maydis* in all samples collected from various fields in the three Agro-ecological regions of Zambia.

FIELD	MEAN	SAMPLE SIZE
CH1	71.481a *	108
CH2	72.667b	105
CH3	75.000c	118
CH4	75.981d	107
CH5	76.486d	111
KB1	75.050c	101
KB2	74.909c	110
KB3	76.00d	105
KB4	76.373d	102
KB5	75.140d	107
KB6	73.627e	102
KB7	76.122d	98
KB8	78.411f	107
LV1	73.761g	109
LV2	73.861h	101
LV3	80.459I	109
LV4	74.779j	113
ND1	77.798f	109
ND2	74.712j	104
ND3	77.273f	110
ND4	83.125I	112
ND5	79.732k	112
ND6	77.982f	109
TOTAL	76.152	2469

*Means followed by the same letter are not significantly different at $P \leq 0.05$ probability level using Least Significant Difference for separation of means.

APPENDIX XIV: Analysis of variance of length of conidia by site.

SOURCE	DEGREE OF FREEDOM	SUM OF SQUARE	MEAN SQUARE	F value	P
Between	3	5600.77	1866.92	26.35	0.0000
Within	2465	174646	70.8503		
Total	2468	180247			

APPENDIX XV: Analysis of variance of length of conidia by region.

SOURCE	DEGREE OF FREEDOM	SUM OF SQUARE	MEAN SQUARE	F value	P
Between	2	4992.82	2496.41	35.13	0.0000
Within	2466	175254	71.0681		
Total	2468	180247			

APPENDIX XVI: Analysis of variance of width of conidia by field.

SOURCE	DEGREE OF FREEDOM	SUM OF SQUARE	MEAN SQUARE	F value	P
Between	22	3.94265	0.17921	2.98	0.0000
Within	2446	147.200	0.06018		
Total	2468	151.143			

APPENDIX XVII: Means of width (μm) of conidia of *Cercospora zeae-maydis* collected from various fields in the three Agro-ecological regions of Zambia.

FIELD	MEAN	SAMPLE SIZE
CH1	10.009a*	108
CH2	10.000b	105
CH3	10.000b	118
CH4	9.9813c	107
CH5	10.000b	111
KB1	10.010a	101
KB2	9.9727c	110
KB3	9.9905b	105
KB4	9.9902b	102
KB5	10.000b	107
KB6	9.9902b	102
KB7	9.9898b	98
KB8	9.9813c	107
LV1	9.8257d	109
LV2	9.9802c	101
LV3	9.9174d	109
LV4	9.9381e	113
ND1	10.000b	109
ND2	10.019a	104
ND3	10.000b	110
ND4	9.9911b	112
ND5	9.9911b	112
ND6	10.000b	109
TOTAL	9.9814	2469

*Means followed by the same letter are not significantly different at $P \leq 0.05$ probability level using LSD for separation of means.

APPENDIX XVIII: Analysis of variance of width of conidia by site.

SOURCE	DEGREE OF FREEDOM	SUM OF SQUARE	MEAN SQUARE	F value	P
Between	3	2.39070	0.79690	13.21	0.0000
Within	2465	148.752	0.06035		
Total	2468	151.143			

APPENDIX XIX: Analysis of variance of width of conidia by region.

SOURCE	DEGREE OF FREEDOM	SUM OF SQUARE	MEAN SQUARE	F value	P
Between	2	2.37061	1.18530	19.65	0.0000
Within	2466	148.772	0.06033		
Total	2468	151.143			

APPENDIX XX: Analysis of variance of number of septa of conidia by field.

SOURCE	DEGREE OF FREEDOM	SUM OF SQUARE	MEAN SQUARE	F value	P
Between	22	605.766	27.5348	17.90	0.0000
Within	2446	3762.66	1.53829		
Total	2468	4368.42			

APPENDIX XXI: Means of number of septa of the conidia of *Cercospora zeaemaydis* collected from various fields in the three Agro-ecological regions of Zambia.

FIELD	MEAN	SAMPLE SIZE
CH1	7.5370a*	108
CH2	7.5429a	105
CH3	7.4322b	118
CH4	7.8411cefh	107
CH5	8.1802d	111
KB1	7.7723eg	101
KB2	7.8364cefh	110
KB3	7.8095cefh	105
KB4	8.0588cd	102
KB5	7.9720d	107
KB6	7.8725cfh	102
KB7	8.0612d	98
KB8	8.3458ij	107
LV1	7.7064g	109
LV2	7.6139ag	101
LV3	7.8073hg	109
LV4	7.7257g	113
ND1	8.2202d	109
ND2	7.8846fh	104
ND3	8.3455ij	110
ND4	9.5268j	112
ND5	9.1250j	112
ND6	8.5505j	109
TOTAL	8.0381	2469

*Means followed by the same letter are not significantly different at probability level $P \leq 0.05$ using the LSD for separation of means.

APPENDIX XXII: Analysis of variance of number of septa of conidia by site.

SOURCE	DEGREE OF FREEDOM	SUM OF SQUARE	MEAN SQUARE	F value	P
Between	3	332.701	110.900	67.74	0.0000
Within	2465	4035.72	1.63721		
Total	2468	4368.42			

APPENDIX XXIII: Analysis of variance of number of septa of conidia by region.

SOURCE	DEGREE OF FREEDOM	SUM OF SQUARE	MEAN SQUARE	F value	P
Between	2	310.096	155.048	94.21	0.0000
Within	2466	4058.32	1.64571		
Total	2468	4368.42			

APPENDIX XXIV: Data used to calculate the net benefits of Early Impact application on maize infected with gray leaf spot caused by *Cercospora zae- maydis*.

Field price of seed at 20 kg/ha	@ K2, 000.00/kg	K40, 000.00
Field price of fertiliser:		
Basal at 200 kg/ha	@ K800.00/kg	K160, 000.00
Top dressing at 150 kg/ha	@ K800.00/kg	K120, 000.00
Field price of fungicide at 1.25L/ha	@ K68, 750.00/L	K85, 927.50
Field price of sprayer	@ K2, 000.00/hour/8 hrs a day	K16, 000.00
Labour to apply fertiliser	@ K5, 000.00/day for 2 days/ha	K10, 000.00
Labour to apply fungicide	@ K5, 000.00/day for 2 days/ha	K10, 000.00

APPENDIX XXV: Total costs that do not vary.

	TREATMENT			
	No spray	Single spray	Two sprays	Three sprays
Cost of seed (k/20 kg)	40,000.00	40,000.00	40,000.00	40,000.00
Cost of fertiliser (Basal kg/200 kg)	160,000.00	160,000.00	160,000.00	160,000.00
Cost of fertiliser (Top dressing kg/150 kg)	120,000.00	120,000.00	120,000.00	120,000.00
Labour to apply fertiliser	20,000.00	20,000.00	20,000.00	20,000.00
Labour to weed	25,000.00	25,000.00	25,000.00	25,000.00
Total costs (k/ha)	365,000.00	365,000.00	365,000.00	365,000.00