INCIDENCE AND SEVERITY OF FROGEYE LEAF SPOT OF SOYBEANS [Glycine max (L.) Merr.] IN AGROECOLOGICAL ZONE II OF ZAMBIA.

BY

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DECLARATION

I, Weston Fredrick Mwase hereby declare that this dissertation represents my own work and that it has not been previously submitted for a degree at this or any other university.

.....

Signature

13/01/98

Date

APPROVAL

This dissertation of WESTON FREDRICK MWASE is approved as fulfilling part of the requirements for the award of the degree of Master of Science in Agronomy (Crop Science) of the University of Zambia.

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DEDICATION

To my mother Mayi Ulemu (Efe) whose insistence turned me towards the light and whose love was an inspiration.

To my wife Esther for her love, patience, endurance and understanding throughout the course of this study which kept us apart for two years when we were barely one year in marriage.

I love you both!

ABSTRACT

Soybean (*Glycine max* (L.) Merr.) is an important crop in Zambia but many foliar diseases pose a serious threat to its successful production in the country. During the last three years frogeye leaf spot disease caused by *Cercospora sojina* Hara has become the most prevalent disease in most parts of Zambia. A survey on farmers' fields under SCS1, Kaleya, Magoye and Hernon-147 cultivars in agroecological zone II of Zambia was carried out during the 1996/97 growing season to determine the incidence and severity of frogeye leaf spot. Disease incidence and severity was studied by monitoring disease progress at two weeks interval from the beginning of January to April. A total of nine fields were surveyed in three provinces namely Central, Lusaka and Southern. Soybean cultivars were evaluated for yield losses resulting from frogeye leaf spot. Field plots of each cultivar were either sprayed twice with benomyl or not sprayed at all.

Nine samples of frogeye leaf spot of soybean, collected from Central, Lusaka and Southern provinces revealed variability among the isolates of *Cercospora sojina* (Cs-01 to Cs-09) in morphology, physiology and virulence. Isolate Cs-08 had the greatest conidial length and Cs-07 had the smallest conidial length. On the basis of conidial width, isolates were grouped in four categories; and included Cs-04, Cs-05 and Cs-06 (small < 5.0μm) ; Cs-08 (broad 6.0-6.5μm) ;Cs-01, Cs-02, Cs-03, Cs-07 and Cs-09 (very broad > 7.0μm) .

After the third day of growth on Carrot leaf decoction agar isolates Cs-04, Cs-07, Cs-08 and Cs-09 produced the largest colony diameters while isolates Cs-02 and Cs-06

had the slowest rate of growth. Growth rate of isolates on Potato dextrose agar and Sabouraud dextrose agar did not show any significant differences ($P \leq 0.05$) throughout the period of culturing. Pathogenicity and virulence of the isolates tested in green house grown plants at four weeks showed that isolates Cs-02 and Cs-08 were the most virulent and produced lesions with a mean diameter of 5.1 mm fourteen days after inoculation. The least virulent isolates were Cs-04, Cs-05 and Cs-05 which produced lesions of 2.0 mm diameter or less.

Results obtained from these studies showed that the incidence of frogeye leaf spot was highest in Southern province (5.1), followed by Lusaka province (4.9) while Central province had the lowest disease incidence (0.3) basing on the 1-9 disease rating scale. Incidence and severity increased with time and varied depending on weather parameters and susceptibility of cultivars to the disease. Yield reduction was greatly influenced by the severity of the disease. Yield losses due to frogeye leaf spot occurred through a reduction in seed size. It is believed that differences in virulence of the isolates contributed to the observed variation in incidence and severity of the disease at different locations.

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LIST OF ABBREVIATIONS

1. AUDPC Area under disease progress curve
2. CLDA Carrot leaf decoction agar
3. Cs *Cercospora sojina*
4. CV Coefficient of variation
5. IITA International Institute of Tropical Agriculture
6. LSD Least significant difference
7. µm Micrometre
8. PDA Potato dextrose agar
9. SDA Sabouraud dextrose agar
10. WP Wettable powder

1.0 INTRODUCTION

Soybean [*Glycine max* (L.) Merr.] is the world's most important oil seed and grain legume crop. The origin of soybean is thought to be from *Glycine ussuriensis* Regel and Maack which is a slender, prostrate wild twining legume of Eastern Asia and *Glycine tomentosa* Benth.(L.) of Southern China (Purseglove, 1968). Most literature document China as the centre of origin (Hume *et al.*,1985). The crop has been cultivated in China since the written documents came in existence. The crop has also been an important food for a long time in Manchuria, Korea and Japan. As a crop soybean has been introduced into tropical countries during this century.

Soybean is a relatively new crop in Zambia and because of the agronomic research, plant protection, utilization and processing work, its cultivation has become a popular and reliable venture (Wellving, 1984). It has been included with sunflower, groundnuts and cotton seed as important sources of oil. Soybean has a high protein content of 40%, carbohydrate 30% and 392 calories of energy per 100 grammes; it has the most balanced protein amongst crops. Thus it is an ideal food for combating malnutrition in this country. The crop is easy to grow and has beneficial effects on the soil and this has led to its inclusion in maize, wheat and other cereal based rotational programmes (Javaheri and Wynae, 1985). Zambia is among the most suitable countries in Africa for soybean production, and with the introduction of self-nodulating cultivars such as Magoye and Hernon-147, many small scale farmers have increasingly been involved in the production of soybean as a cash and a food crop.

Bread has become the 'fast food' of Africa, Zimbabwe and Nigeria in particular. Approximately 1.5 million metric tonnes of wheat was imported into Nigeria in 1983. If Nigeria were to replace 10% of the wheat flour with full fat soybean wheat flour, as is being done in Zambia, then the requirement would be 150,000 metric tonnes for wheat replacement alone. Such a decision would increase the food value of the bread which is consumed by many school children that have high protein requirements and at the same time reduce the use of foreign exchange which is in critical supply. Malawi, the Democratic Republic of Congo and Republic of Benin are introducing soybeans into the rural sector. Child Health Centres and Missionaries of various denominations have introduced the crop as a medicinal food to treat and prevent protein malnutrition, it is also used as a weaning food (Anonymous, 1983).

Diseases pose a major problem to soybean production in the tropics (Hartman *et al.*,1991). Research at International Institute of Tropical Agriculture (IITA) in Nigeria and elsewhere in the world have revealed that soybean has several constraints in the tropics.These problems include foliar diseases, poor seed viability and the requirement of specific microbial inoculants for effective nitrogen fixation (IITA, 1987). Although the climate of Zambia is suitable for soybean production, the crop is susceptible to a number of diseases. The most common soybean diseases in Zambia are bacterial blight caused by *Pseudomonas glycines* (L.), Red leaf blotch incited by *Pyrenochaeta glycines* (L.), bacterial pustules caused by *Xanthomonas phaseoli* (L.) and Frogeye leaf spot which is caused by *Cercospora sojina* Hara (Datnoff *et al.*,1987). Frogeye leaf spot, also known as *Cercospora* leaf spot, was

first documented in Zambia on leaves of soybeans in 1978 (Javaid and Ashraf, 1978). It was, however, reported as a minor disease. During the last three growing seasons, the disease was a problem at Mpongwe in the Copperbelt Province, Northern Province and some parts of Lusaka (Anonymous, 1995).

Yield loss due to a particular disease depends, *inter alia*, on the incidence and severity of the disease. An average loss of 15% is reported due to frogeye leaf spot, but in Nigeria where the disease is very common, yield losses of up to 60% and in Brazil losses of up to 80% have been documented (Sinclair and Backman, 1989). In most developing African countries population growth is exceeding food production and consequently per capita food intake is declining. One of the limiting factors in attaining food self-sufficiency in Africa is crop diseases hence protein intake has drastically declined in the last decade (IITA, 1983). Diseases, such as Red leaf blotch and Frogeye leaf spot pose major threats to soybean production in Zambia. Since the reappearance of Frogeye leafspot during the last three growing seasons as a major disease, it has been a serious problem in some parts of the country. To establish the seriousness of the disease it was considered important to study its incidence, severity and the yield losses associated with it. Disease severity varies greatly at different locations, the variations might be due to differences in environmental conditions, especially the degree of drought stress, or variability in the causative pathogen. Due to pathogen variability, the virulence at different locations and different cultivars may vary as well. Measured differences in pathogenicity and virulence among isolates of *Cercospora sojina* Hara (L.) was carried out to test for variability of the pathogen.

The relationship between proportion of plant units diseased (incidence) and the amount of plant tissue infected by the disease (severity) is a valuable tool for disease assessment and management (Seem and Gilpatrick, 1980). In most cases it is considerably easier to determine the incidence of a disease rather than its severity; nevertheless, disease severity often is the preferred measurement because it describes the relative area of plant tissue infected. Although the area of infected tissue is the most commonly used measurement of severity, other measurements, such as number of lesions can also be used, especially when lesions are relatively uniform in size (Seem and Gilpatrick, 1980).

In this pathosystem involving several *Cercospora sojina* isolates, one must distinguish between pathogenicity and virulence. Here, pathogenicity is defined as the ability to cause disease. Virulence is defined as the relative ability of a pathogen to cause damage on a specific host under certain environmental conditions. Rate of spread and percent mortality of a given infection are both considered as measures of virulence. Virulence in previous inoculation trials with *Cercospora* species has commonly been based on one or more of the following; defoliation, number of lesions per plant, size of lesions, rapidity of spread and time to death (Omdal *et al.*,1995).

Although significant losses in soybean yield have often been attributed to frogeye leaf spot (Southern Soybean Disease Workers, 1988) a definitive relationship between disease severity and yield has not been established in Zambia. Furthermore, no accurate identification of *Cercospora sojina* has been reported hence yield losses were based on symptoms only.

The objectives of this study were:

i. To determine the incidence of Frogeye leaf spot in agroecological zone II of Zambia.

ii. To determine the relationship between severity of Frogeye leaf spot and yield loss.

iii. To determine variability, pathogenicity and virulence of *Cercospora sojina* isolates from different locations and cultivars.

2.0 REVIEW OF LITERATURE

The fungus causing Frogeye leaf spot soybeans was described as *Cercospora sojina* by Hara (1915) in Japan. Miura (1921), in a brief mycological note, published a description of a Cercospora leaf spot disease of soybean which he found at Tumeling in Southern Manchuria. He evidently was not aware of the earlier report by Hara because he ascribed it to a new fungus which he named *Cercospora daizu* (L.) (Miura, 1921). In the United States both species names have been used. Frogeye leaf spot was also observed in Indiana in 1939 in the extreme Southern part of the state by Probst, A.H. and identified by Caldwell of the Purdue Agricultural Experiment Station (Athow and Probst,1952). The disease was of minor importance until 1947 when it occurred in epiphytotic proportions in the large soybean growing areas of Southwestern Indiana. It continued as the most important soybean disease through the 1949 season after which incidence was reduced greatly by widespread use of the resistant varieties Lincoln and Wabash. A marked difference in susceptibility among

soybean varieties was observed. Field observations indicated that varieties Lincoln and Wabash had a high degree of resistance whereas Gibson and Patoka were very susceptible (Phillips and Boerma, 1981).

Horn *et al.*, (1975) evaluated soybean yield losses resulting from stem and foliar diseases following green house inoculation of three soybean cultivars and reported that *Corynespora cassicola* (Berk. and Curt.) Wei caused the greatest yield losses following in decreasing order by *Cercospora sojina* Hara and *Diaporthe phaseolorum* (Cke and Ell.) Sacc. Var.soja (Lehman), Wehm. They concluded that yield decreases were more commonly related to smaller seed size than to numbers of seeds harvested. Thus higher yields and seed weight/1000 seeds were obtained when soybean was treated with benomyl as opposed to plants which received inoculation of *Cercospora sojina* but were not treated with benomyl.

Backman *et al.*, (1979) evaluated several field-grown naturally infected cultivars of soybeans and determined that significant increases in yield and significant reductions in several foliar diseases resulted from application of fungicides. No attempt was made to quantify the yield loss contributions of the individual diseases.

Yorinori and Sinclair (1982) reported that all stages of the soybean plants, if exposed to the wet season, develop severe frogeye leaf spot disease. This depends on topography and location. The need for resistant cultivars in such areas was suggested by Laviolette *et al.,* (1970) and Yorinori and Sinclair (1982).

There is high variability in pathogenicity, colony characteristics, conidial size and number of septa per conidium (3 to 13) among isolates of *Cercospora sojina* from different regions and cultivars (Yorinori and Sinclair, 1982). Cultivars found to be resistant in the USA were susceptible in other countries. They also reported the effects of *Cercospora sojina* on defoliation and seed weight of soybeans. Twenty nine cultivars were inoculated with 10 isolates of *C. sojina*. Comparisons made among percent defoliation and seed weight reduction by *C. sojina*, *C. kikuchii* and *Phomopsis* species showed high defoliation and greatest seed weight reduction due to *C. sojina*.

Manandhar and Sinclair (1982) documented highest severity of frogeye leaf spot in mid hills, least in plains and intermediate severity at high hills of Nepal. Indigenous cultivars of soybeans were more susceptible to *C. sojina*.

Frogeye leaf spot is reported as a common disease in Kaduna and plateau states of Nigeria. The disease was first observed in the country in 1981 (IITA, 1987) and has since become widespread in most of the soybean growing regions of the country. Dashiell and Akem (1991) reported differences in levels of frogeye leaf spot development on all nonsprayed soybean cultivars. Disease severity for nonsprayed cultivars ranged from 0.5 to 4.5 on a 0-5 rating scale. Benomyl treatment produced a range of reactions within two locations. A highly significant negative correlation between the severity of Frogeye leaf spot and grain yield was reported. A loss of 65% was obtained for the most susceptible cultivar, 17% for the moderately

susceptible and 5% for the least susceptible cultivar. Seed yields of resistant cultivars were virtually unaffected by treatment with benomyl. There was, however, a significant difference in weight of seed from sprayed and nonsprayed plots of the resistant cultivar.

Phillips and Boerma (1981) investigated the threat of *C. sojina* races to soybeans in Southeastern United States. Several fields planted with Clark and Wabash cultivars, which were previously resistant to the frogeye leaf spot, became heavily infected. Results of experiments on controlled inoculation of selected cultivars in the greenhouse with isolates from these fields and with isolates obtained in 1949 indicated varietal specificity. The results clearly demonstrated varietal differences in susceptibility to the two isolates, hereafter referred to as race I and II. The two races of fungus differ slightly in the way they are cultured as well as in their morphological characteristics.

Race I produces a dense grey mycelial growth on PDA and race II is characterised by a dark, more sparse, appressed mycelial growth. A sufficient number of isolates of either race have not been observed to ascertain whether this characteristic is stable. Spore shape and size vary with environmental conditions. Length of conidia, a variable character, were essentially the same in both races. However, Chupp (1953) considered width as an important criterion for classification; conidia of race II were wider by 1.0-1.5µm than those of race I, although the ranges were about the same. The conidia of race I were obclavate-cylindrical with obconically-truncate

bases and acute to obtuse tips. Chupp (1953) considered the shape of the conidial base as the most dependable character for species differentiation, but he realised that only a few base types were distinct enough to make unfailing classes among the species.

2.1GROWTH OF *CERCOSPORA SOJINA* ON VARIOUS MEDIA

Crane and Crittenden (1967) reported the extent of mycelial growth of *C. sojina* on various media in the laboratory in which bacto-agar, potato dextrose agar, seed cotyledon and embryo agar and Czapek's agar were used. A plug of uniform size of *C. sojina* mycelium was obtained from culture maintained on bacto-agar and transferred to petri plates whose colony diameter measurements were taken after 7, 14 and 21 days. Measurements of colony diameter taken at 7-days intervals showed little difference between growth on potato dextrose agar and agar prepared from seed cotyledon and embryo agar, however after 14 days the colony diameter on cotyledon and embryo agar was considerably greater than on PDA. The same difference was also recorded after 21 days.

The isolates of *C. sojina* studied by Matsumoto and Tomoyasu (1925) sporulated on raisin decoction agar but not on other 13 decoction agars or on three defined liquid media. Kilpatrick and Johnson (1956) and Lyda *et al.*, (1979) induced sporulation of the fungus by using Carrot leaf decoction agar but Vathakos and Walters(1978, 1979) failed to verify the results .

Yeh and Sinclair (1979) compared the growth and sporulation of five isolates of *Cercospora sojina* on four media: PDA, Carrot leaf decoction agar, V-8 juice agar and dead soybean plant tissue agar (DSPT). All isolates sporulated on all media tested under alternating 12 hour of light and dark, but sporulation was sparse under continuous darkness. The average conidia size did not differ among isolates; however, the range within each isolate was wide.

2.2 SYMPTOMATOLOGY OF FROGEYE LEAF SPOT

Frogeye leaf spot is primarily a foliar disease even though stems, pods and seeds may also be infected. Minute, reddish brown, circular and angular spots first appear on the upper leaf surface. The central parts become olive-grey or ash grey and is surrounded by a narrow, dark reddish-brown border. The lesions vary in size from <1 mm to 5 mm (Sinclair and Backman, 1989). On the lower leaf surface, spots are darker brown or grey. Clusters of dark conidiophores develop in the centre of each lesion, mostly on the underside of the leaf. Lesions on the pods are circular to elongate, slightly sunken and reddish-brown. As lesions enlarge, the centres become brown to light grey. The pathogen frequently grows through the pod wall into maturing seeds (Sinclair and Backman, 1989).

2.3 MORPHOLOGY AND PHYSIOLOGY.

Conidiophores of *C. sojina* on host arise in fascicles of 2 to 25 from a thin stroma.

Conidiophores are dark to light brown and vary in length between 52.0-120.0 X 4.0-6.0 µm. They are one to four septate with prominent bends and conidial scars. The conidia are borne on the tips of conidiophores and are pushed as the conidiophores continue their indeterminate growth. The conidia are 0-10 septate, hyaline when young, elongate to fusiform and taper towards the tip. The base of a conidium is usually rounded, bearing circular scars marking the attachment to the conidiophore. On infected leaves the conidia are 24.0-108.0 X 3.0-9.9 µm but a size range of 40.0-60.0 X 6.0-8.0 µm is most common. The size and shape of conidia and conidiophores vary with the substrate on which the fungus grows (Sinclair and Backman, 1989).

Conidia germinate within an hour in tap water, producing one to several germ tubes from the end cells. In culture, conidia frequently germinate *in situ* forming short germ tubes which in turn, produce secondary conidia. *Cercospora sojina* grows and sporulates poorly on meat media. Sporulation areas can be found *in vitro* by some isolates and these sporulate best on V-8 juice agar (Sinclair and Backman, 1989). On water agar, sparse development of aerial hyphae occurs in about one week and-smoky-brown conidiophores with conidia are subsequently formed.

Colonies of *C. sojina* develop on potato dextrose agar as compact mycelial mats in alternating light and dark bands giving the appearance of concentric rings, with the surface convoluted or folded radially.

2.4 DISEASE CYCLE AND EPIDEMIOLOGY

Cercospora sojina survives as mycelium in infected seeds and infested debris (Sinclair and Backman, 1989). Germination of infected seed is reduced slightly. Infected seeds that germinate may produce weak, stunted seedlings with lesions on the cotyledons. Sporulations on the cotyledonary lesions provide primary inoculum for infection of the young leaves. The conidia are carried to short distances by air currents and with favourable conditions, secondary leaf, stem and pod infections arise throughout the season. Young leaves are more rapidly infected, and with adequate moisture, leaves become infected as they develop until all the leaves on a plant are infected (Sinclair and Backman, 1989).

2.5 CONTROL MEASURES

The pathogen is seed-borne and is also carried to the next cropping season in crop residue (Sinclair and Backman,1989). The control measures of frogeye leaf spot include: Growing resistant cultivars; planting high-quality and pathogen-free seeds or rotating soybean with other crops for two years. The disease can also be controlled by foliar spray of fungicides such as benomyl (benlate 50 WP).

3.0 MATERIALS AND METHODS

3.1DISEASE INCIDENCE AND SEVERITY IN THE FIELD

Surveys of frogeye leaf spot of soybean were conducted from January to April, 1997

in three provinces : Central (Kabwe), Lusaka (south and west), and Southern (Mazabuka) which fall under agroecological zone II (Fig.1). In order to provide a meaningful estimate of the disease for each province, five fields were randomly selected from each province. Disease incidence was recorded four times within the season at an interval of two weeks. From each field the incidence was recorded by randomly selecting a group of 10 plants at five places within a standardized plot, four in the corners and one at the centre. A portion of 10-m^2 area of each field was used in disease rating. In each place the total number of healthy and diseased leaves was recorded under 0- 9 grades from three randomly selected branches.

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

The Percent Disease Incidence (PDI) was calculated as per FAO methods of plant disease assessment (FAO,1971)

$$PDI = \frac{\text{Sum of numerical values}}{\text{Total No. of leaves observed X Maximum grading (9)}} \times 100$$

The disease severity scale was based on number of spots per leaflet, thus individual leaves represented the sampling unit and they were rated for the absence or presence of frogeye leaf spot. *Cercospora* severity was measured as the percentage of the total leaf area diseased using a modified Horsfall-Barrat rating system (1945). The disease severities recorded at different time intervals were converted to area under the disease progress curve (AUDPC) according to the formula presented by Shaner and Finney (1977) where:

$$AUDPC = \sum_{i}^{n} [(Y_{i+1}+Y_i)/2] [X_{i+1}-X_i] \text{ where}$$

Y_i= disease severity (%) at the i^{th} observation

X_i= time (days) at the i^{th} observation

n= Total number of observations.

The Disease Severity Index (DSI) is the average of all ratings for each plot within an experimental replicate for each time the ratings were made. Ten plants were usually rated from each of the five randomly chosen places of each field.

Figure 1: Surveyed sites for Frogeye leaf spot of soybeans in Lusaka, Central and Southern Provinces of Zambia

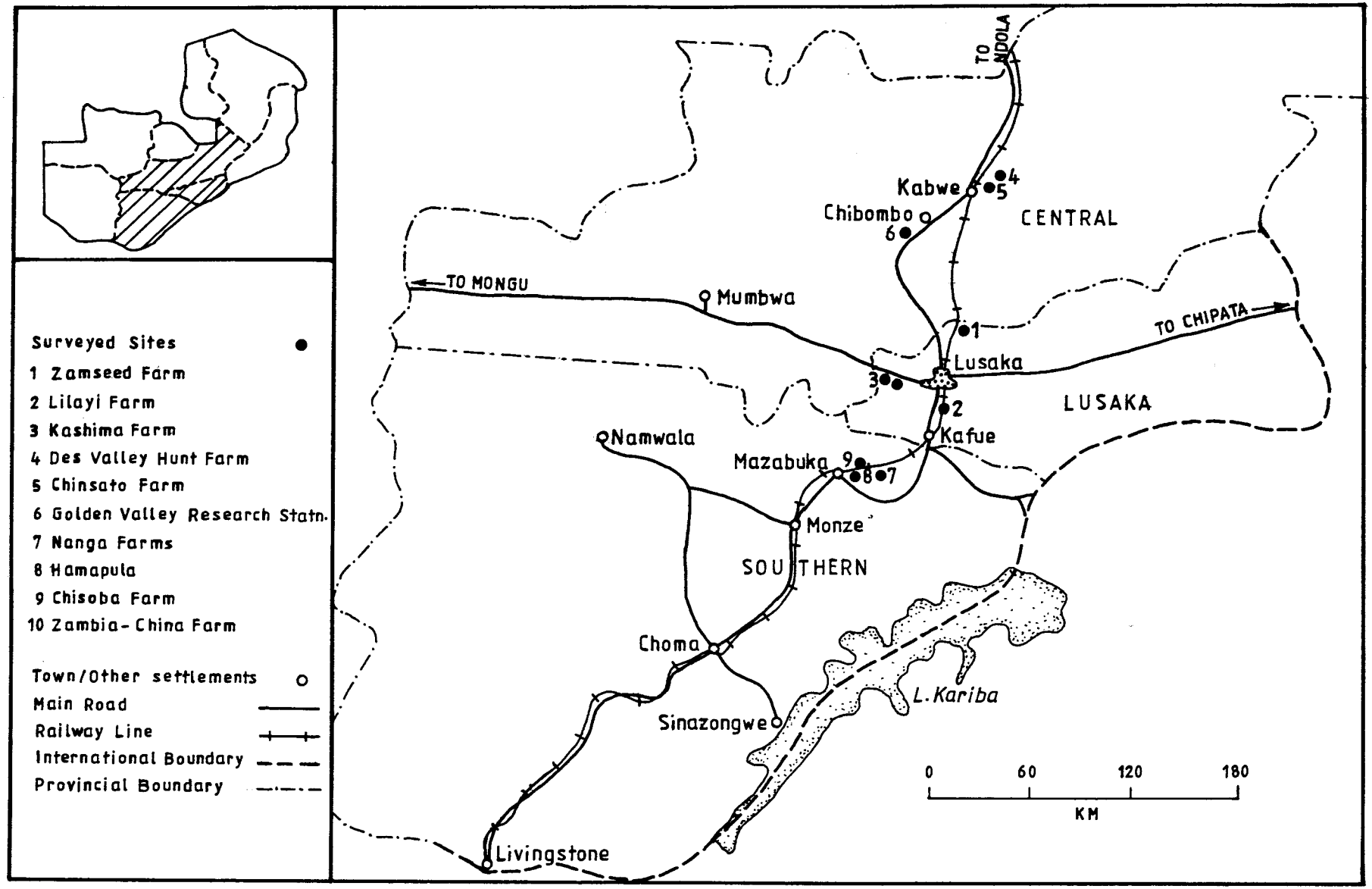


Fig. 1 : Surveyed sites for Frogeye leaf spot of soybeans in Lusaka, Central and Southern provices of Zambia.

Assessment of yield loss due to frogeye leaf spot was conducted at Zamseed farm, Lusaka. Four soybean cultivars namely Hernon-147, Kaleya, Magoye and SCS1 were planted on a fertile, well-managed sandy-loam soil which had been planted with maize the previous cropping season. The plants were grown in rows of 8 m long and spaced at 1 m width at the rate of 40 seeds per metre. Plots were either sprayed twice or not sprayed at all with benomyl. The first spray was done at R1 and the final spray at R3 stage of growth. Sprayed plots were given a concentration of 0.5 kg ha^{-1} benomyl to control the pathogen. Each treatment was replicated four times in a completely randomised block. The four soybean cultivars were the main plots and the fungicide treatments the subplots in a split-plot design. Disease inoculum was from natural sources. The fungicide was first applied at the V4 growth stage (Fehr *et al.*,1971) and two weeks later. A backsprayer (Knapsack Sprayers CP3: ICI, Fernhurst, Haslemere, Surrey, England) delivering 200*l* ha^{-1} was used. The disease ratings were made every 14 days beginning at V5 until harvest maturity (R8) stages. Disease severity was measured as the percentage of the total leaf area infected using the Horsfall-Barrat rating system. Rating was done on 10 plants taken at random from each net plot (three middle rows with 1 m discarded from each side of the row). Area under the disease progress curve was also determined using the formula by Shaner and Finney (1977). In field studies the presence of other pathogens other than the one being studied may affect the results. Therefore the occurrences of Red leaf blotch and bacterial blight, were recorded .

At the end of the season, plots were harvested by hand, mechanically threshed, cleaned and air-dried. A 6 m section of the middle three rows of each plot were harvested to determine yield loss. Yields were measured as kg per plot and converted to kg ha^{-1}. Harvested seeds were further dried at 32° C for 24 hrs to 13 % moisture. The moisture content of seeds for each plot was measured using a seed moisture meter (Steinlite electronic Moisture Tester: SeedBuro Equipment Co., Chicago, IL 60607). The 300- seed weights were determined and expressed in grammes. Percentage yield reduction was calculated using the following formula (Pataky and Lim, 1981):

$$\text{Yield reduction (\%)} = \frac{\text{Yield from sprayed plots- Yield from nonsprayed plots X100}}{\text{Yield from sprayed plots.}}$$

Seed weight reductions were similarly determined. Yields of the nonsprayed and sprayed plots were compared by an analysis of variance, and by least significance difference (LSD) tests. The data were analyzed using the Mstatc package (Gomez and Gomez, 1984).

3.2 COLLECTION OF *CERCOSPORA SOJINA* SAMPLES

The isolates of *Cercospora sojina* used in the study were obtained from diseased leaf samples collected from: Central (Kabwe and Chibombo), Lusaka (South and West), and Southern (Mazabuka) Provinces. A representative of three isolates for each province was sampled at the reproductive stage from February to April in 1997.

Records of host cultivar and date of collection were made at the time of sample collection. Infected leaflets were transferred to unused polyethylene bags in a cool box and taken to plant pathology laboratory at the University of Zambia for processing. Samples were stored in a fridge overnight before isolating the fungus the next day. Nine isolates were collected as shown in Table 1.

3.3 ISOLATION OF CERCOSPORA SOJINA

Isolations of *Cercospora sojina* were made from leaf lesions on leaves and it was done by plating surface-sterilised sections of the infected tissue on moist filter paper. The isolates were obtained from different soybean cultivars from various localities over a three month period. Fifty lesions for each isolate were cut with a sterilised scalpel and disinfected by dipping them into 70% alcohol for 15 seconds then into a solution of 1% sodium hypochlorite for 5 minutes, and finally into sterile distilled water. The lesions were then placed on moist filter paper in petri plates 9.0 cm and incubated at 25^0C.

TABLE 1. The localities of collection of samples of *Cercospora sojina* Hara

PROVINCE	CULTIVAR	FARM	ISOLATE CODE
Lusaka	Hernon-147	Zamseed	Cs-01
Lusaka	SCS1	Lilayi	Cs-02
Lusaka	SCS1	Kashima	Cs-03
Central	SCS1	D.V.Hunt farm	Cs-04
Central	Hernon-147	Chinsato	Cs-05
Central	Hernon-147	Chibombo	Cs-06
Southern	SCS1	Nanga	Cs-07
Southern	Kaleya	Hamapula	Cs-08
Southern	Gazelle	Chisoba	Cs-09

Culture plates were examined after one day under a dissecting microscope for the presence of conidia. While observing sporulating lesions with the aid of a stereoscopic microscope, a bit of potato dextrose agar adhering to the tip of a transfer needle was brought into contact with conidia and streaked on PDA plates. Plates were incubated at 25^{0}C for 5 days and thereafter stored in a fridge at 8^{0}C till required for further studies.

3.4 CONIDIAL CHARACTERISTICS AMONG *CERCOSPORA SOJINA* ISOLATES

Measurements of length and width of conidia were made from 15 ml homogenous conidial suspensions prepared by washing conidia from 50 lesions of each isolate which had been induced to sporulate in moist petri dishes. Conidial suspension of

each isolate was transferred to a microscope slide and the length, width, shape and number of septa per conidium were determined using an ocular microscope at 40 X magnification. The measurements of conidial length and width were expressed as a range of means. The shape of each conidium was determined by placing conidia to one of the following four shape categories (Yeh and Sinclair, 1979):

a. Fusiform with acute tips.

b. Cylindrical with acute tips.

c. Cylindrical with obtuse tips.

d. Elongate with acute tips.

3.5 COLONY GROWTH AMONG *CERCOSPORA SOJINA* ISOLATES

Colony growth in isolates of *Cercospora sojina* was studied on three different media; Carrot leaf decoction agar (CLDA), Potato dextrose agar (PDA), and Sabouraud dextrose agar (SDA). All media were sterilised and the pH was adjusted to 6.5 before pouring them into glass petri plates of 9.0 cm diameter . A 4 mm mycelial disc of *C. sojina* was obtained from cultures maintained on PDA and transferred to each petri plate. Cork borer No. 4 was used in making wells on the agar and obtaining the mycelial plugs. Each isolate was replicated three times on each medium following the randomised complete block design. The petri plates were placed in a constant temperature chamber set at 25^0C under 12 hr of alternating light (400 $\mu E/m^2/sec$) and darkness for 9 - 16 days.

The diameter of each colony was measured at 3-day interval for a total growth period of 12 days. Colony colour of isolates was determined using the colour names from Munsell soil colour charts (1975). A pH meter was used to determine the colony pH at the 12th day of culture.

3.6 PATHOGENICITY AND VIRULENCE OF *CERCOSPORA SOJINA* ISOLATES TO SOYBEAN CULTIVARS

Greenhouse grown plants of soybean cultivars Hernon-147, Kaleya, Magoye and SCS1 were used to test for the pathogenicity of *C. sojina*. Hernon-147 and Kaleya are commonly grown by small scale farmers while Magoye and SCS1 are grown by large scale farmers in Zambia. Five seeds of each cultivar were sown in 12-cm plastic pots and were later thinned to three seedlings per pot. Pots were filled with autoclaved sandy loam soil of 6 kg for each pot, autoclaving was done at 15 Psi for 4 hr. Each cultivar was replicated three times and inoculated with the nine isolates of the pathogen. The layout of the plots followed a split plot design and treatments were randomised in replication. The cultivars were used as main plots and isolates as sub plots.

Cultures of the nine isolates of *C. sojina* were maintained on PDA and regular transfers were made until the inoculum was needed. Inoculum was prepared by lightly scraping off the mycelial mats from 7-day old culture plates. They were macerated in 300 ml sterile water in a Waring food blender using contents of 3 plates

per isolate and straining it through a double layer of cheesecloth. Seedlings were inoculated at the V3 growth stage using B-D Yale tuberculin syringes and 25-gauge needles. Inoculation was done early in the evening to prevent prolonged exposure to sunlight and rapid drying of the inocula. Each pot was inoculated with 200 ml of the suspension. The check was inoculated with sterile distilled water. Plants were placed in a moist chamber held for 72 hrs at 25-30°C and later returned to the greenhouse bench. During these experiments the green house temperatures were maintained between 18 and 28°C and the plants were regularly watered. Although relative humidity was not controlled but it was always above 50%. Disease ratings were made 14 days after inoculation. Isolates which caused lesion size of about 2 mm were considered pathogenic. Isolates causing leaf spots >3 mm in diameter and more than 10 leaf spots per plant were considered virulent. Thus the response of cultivars to the isolates of the pathogen was considered on the basis of number of lesions per plant, lesion size at 14 and 21 days following inoculation.

4.0 RESULTS

4.1 Symptoms of frogeye leaf spot of soybeans

The lesions appeared on the foliage beginning from R1 growth stage. They were circular to angular spots varying in size from less than one to six millimetres in diameter. They begun as dark, water soaked spots, with or without a light coloured centre, and later developed into brown spots surrounded by a narrow dark reddish brown margin. As the lesions aged, the central area became ash grey. On the lower surface of leaves, the spots were darker with light to dark grey centres and a thin reddish brown margin.

Lesions on pods were observed at R3 growth stage. They were circular to elongate, slightly sunken, and reddish brown. Older lesions became brown to light with a narrow, dark brown border. Elevated conical lesions developed where the infected membraneous inner lining of the pod was in contact with the seeds.

4.2 Prevalence and incidence of frogeye leaf spot

Frogeye leaf spot incidence and area under disease progress curve (AUDPC) for four soybean cultivars in three provinces of Zambia are presented in table 2. Incidence ratings ranged from 1.3 to 2.9 and from 0.3 to 5.1 for the three provinces and four cultivars respectively. Mean incidence rating was greatest in Lusaka province and lowest in Central province. There was no significant difference ($P \leq 0.05$) between incidence in Lusaka and Southern province, however both provinces

had incidence ratings which were significantly higher than the one for Central province. In all the provinces incidence was greatest on SCS1 cultivar followed by Hernon-147. Incidence of frogeye leaf spot was lowest on Magoye and intermediate on Kaleya. There were no significant differences ($P \leq 0.05$) in frogeye incidence ratings among the four cultivars in Lusaka and Southern provinces. Very severe incidence of frogeye leaf spot was observed on one field in Southern province where the rating reached 5.1 (Fig 2). The AUDPC for Lusaka and Southern provinces were not significantly different although Southern Province had the highest AUDPC of 586.07. Central province had a significantly lower AUDPC value 262.52 than the two provinces. The range of AUDPC for the provinces was 262.52 to 586.02 and 38.15 to 1095.40 for the four soybean cultivars.

Figure 2: Symptoms of Frogeye leaf spot of soybeans from one of the surveyed sites.

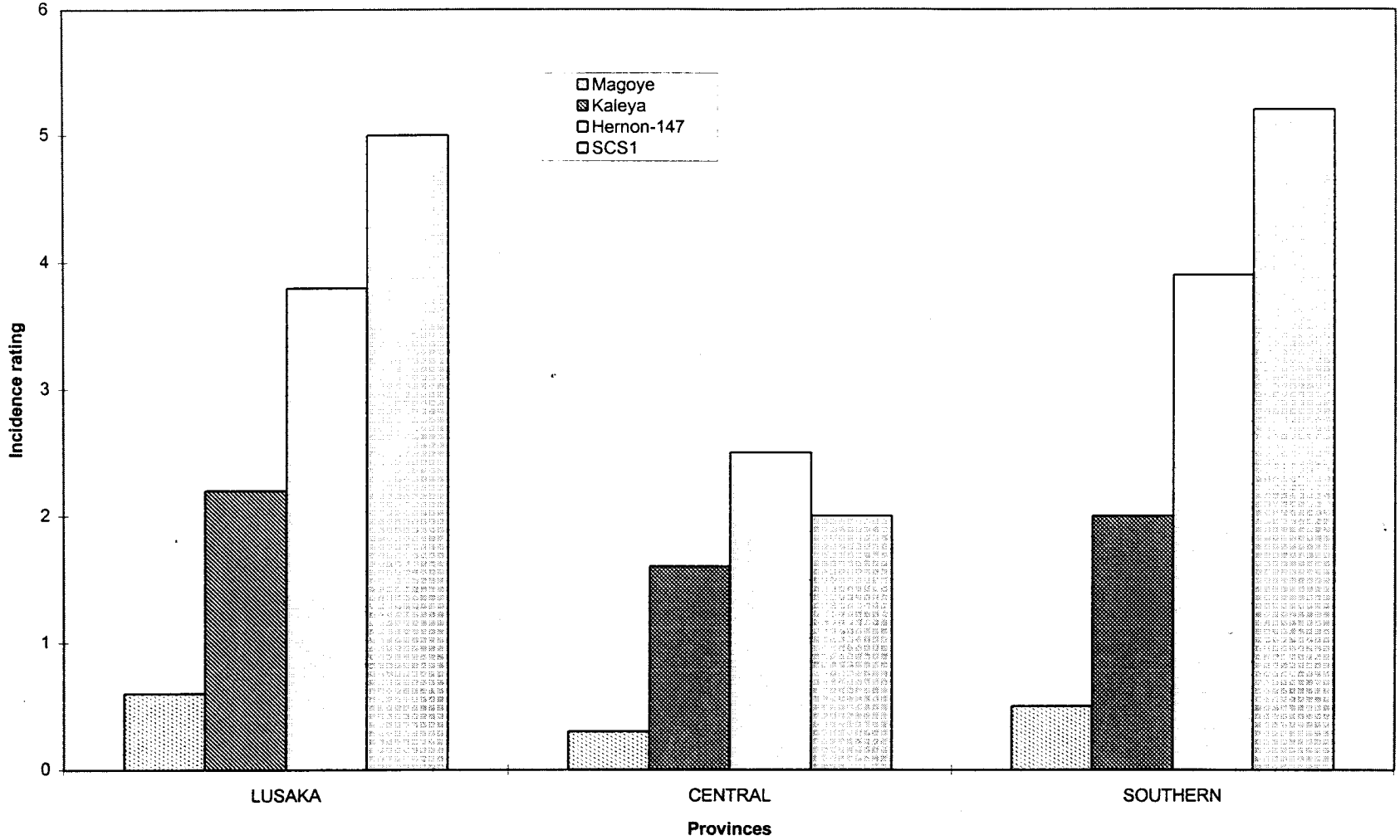


Fig. 3: Incidence rating of Frogeye leaf spot in three provinces of agroecological zone II of Zambia

Table 2: Mean disease incidence ratings and area under disease progress curve (AUDPC) of four soybean cultivars in three provinces of Zambia[y]

Province	Cultivar	Disease incidence	AUDPC
Lusaka	SCS1	4.9 [a*]	1038.79 [a*]
	Hernon-147	3.7 [b]	785.91 [b]
	Kaleya	2.2 [c]	446.67 [c]
	Magoye	0.6 [e]	69.50 [e]
	Mean	2.9	585.22
Central (Kabwe)	SCS1	1.8 [cd]	380.00[cd]
	Hernon-147	1.5 [d]	318.10 [d]
	Kaleya	1.6 [d]	313.83 [d]
	Magoye	0.3 [e]	38.15 [e]
	Mean	1.3	262.52
Southern	SCS1	5.1 [a]	1095.40 [a]
	Hernon-147	3.8 [b]	817.27 [b]
	Kaleya	1.9 [cd]	384.05[cd]
	Magoye	0.5 [e]	47.57 [e]
	Mean	2.8	586.07

* Means followed by the same letter in each column were not significantly different at 0.05 probability level according to Duncan Multiple Range Test.

[y] Average of four replications.

4.3 Frogeye leaf spot severity and yield loss

Severe levels of frogeye leaf spot developed on all nonsprayed soybean cultivars except on Magoye. Fungicide treatment produced a range of disease severities among the cultivars. Frogeye leaf spot was significantly more severe on nonsprayed SCS1 and Hernon-147 cultivars than on Kaleya. Disease severity for nonsprayed cultivars at the R1 to R4 growth stages ranged from 5.3% to 50.2% while that of the sprayed cultivars ranged from 5.0% to 11.6% (Table 3). The disease occurred at a significantly lower rate on Magoye regardless of no fungicide treatment on the control. SCS1 registered the highest frogeye leaf spot severity (50.23%) on the nonsprayed plots (Fig.3).

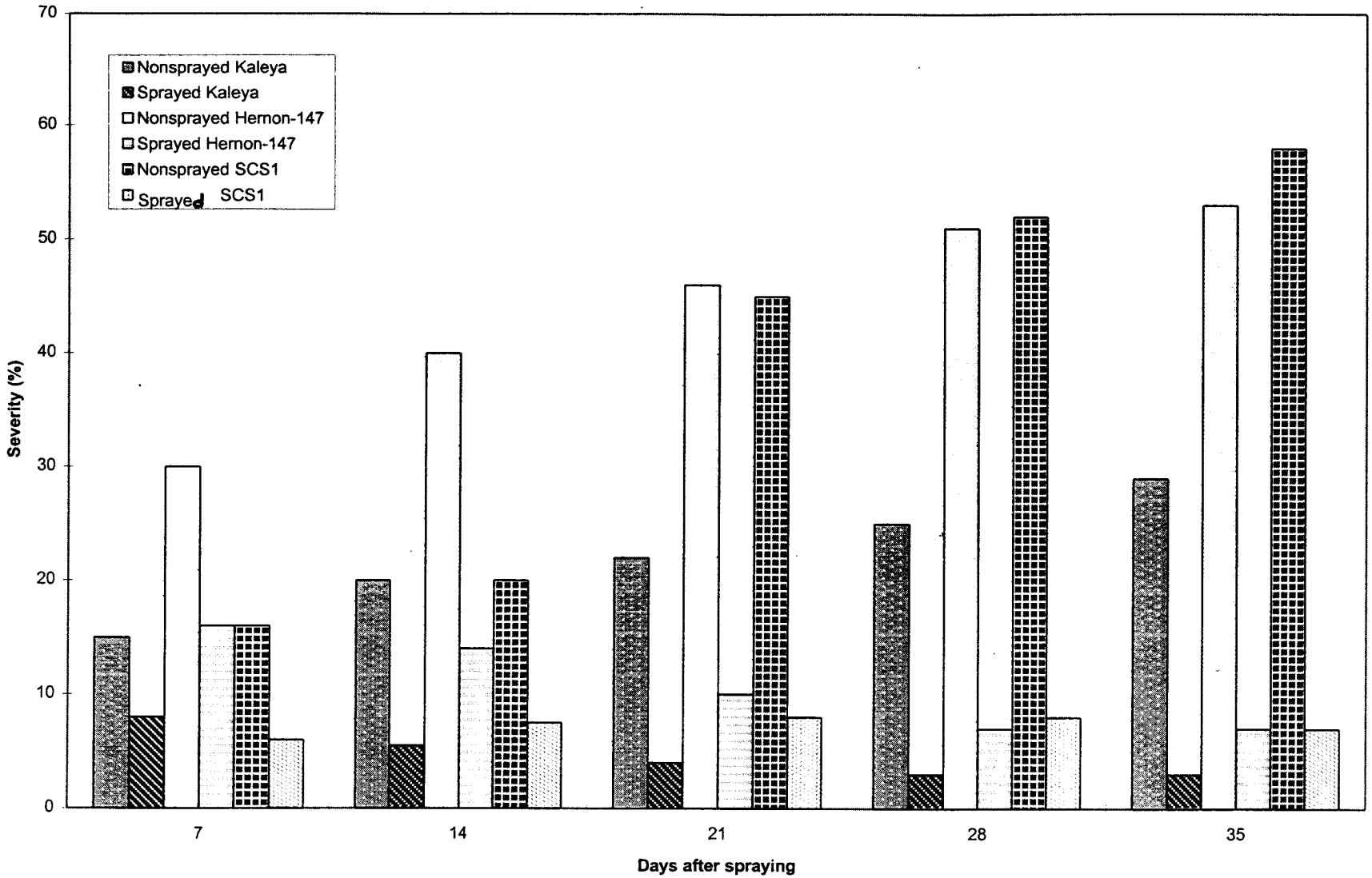


Fig. 4: Frogeye leaf spot severity for benomyl-sprayed and nonsprayed soybean cultivars

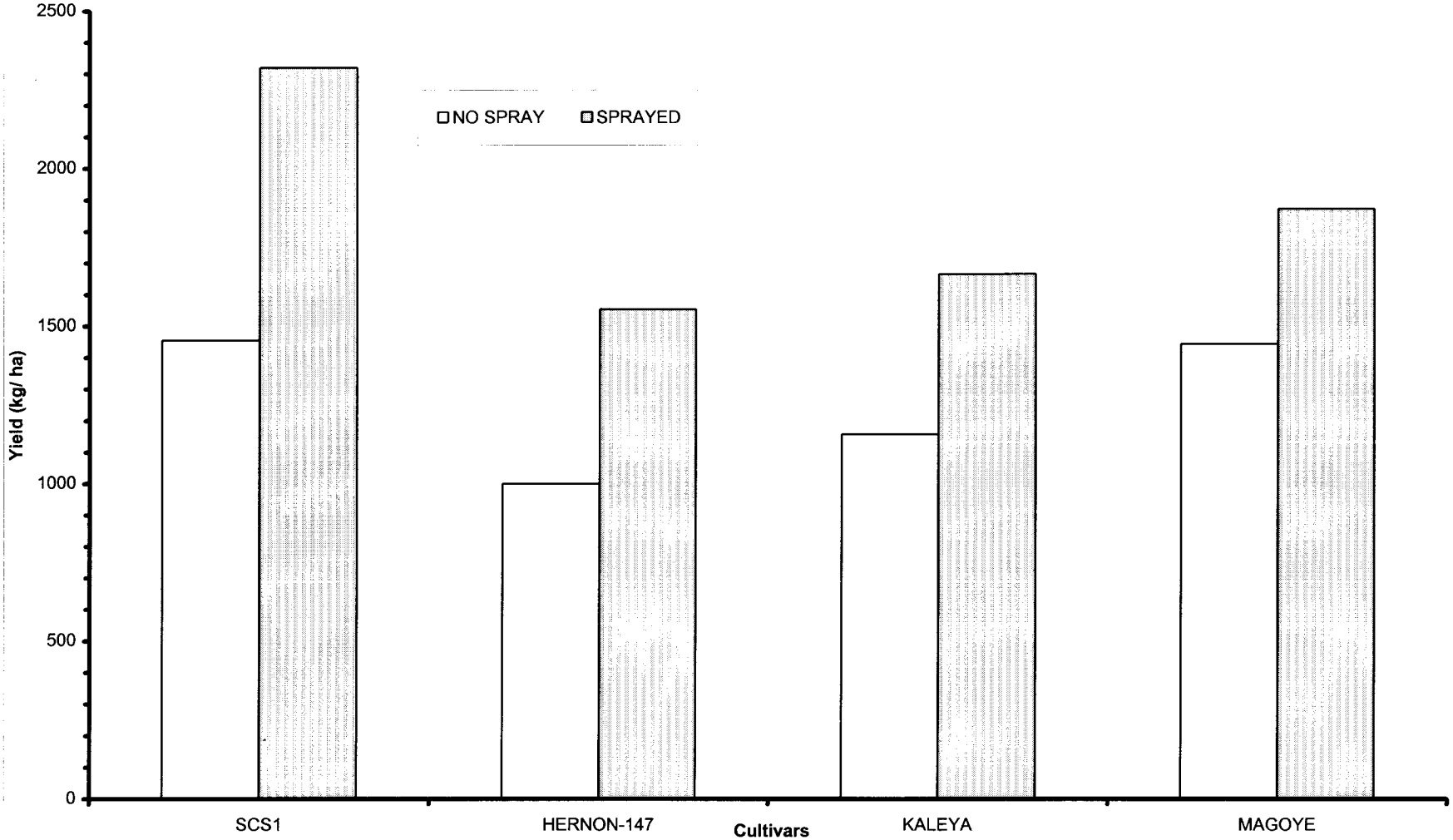


Fig. 5: Yield of benomyl-sprayed and nonsprayed soybean cultivars

The AUDPC values were lowest in the sprayed plots compared to the nonsprayed plots. Kaleya and Magoye had significantly lower AUDPC values ($P \leq 0.05$) while SCS1 and Hernon-147 had the highest values of 1048.0 and 998.2 respectively. There were significant differences in AUDPC values between SCS1 and Hernon-147 benomyl-sprayed and nonsprayed plots.

Yield and 300-seed weight differed significantly ($P \leq 0.05$) between sprayed and nonsprayed plots (table 3). When cultivar yields were compared, nonsprayed plots showed significant yield reductions of 35.6, 26.7, 30.5 and 37.2% for Hernon-147, Magoye, Kaleya and SCS1 respectively. The yield of SCS1 was significantly higher than that of Hernon-147 for both sprayed and nonsprayed plots (Fig 4). Reductions in 300-seed weight were significant for Hernon-147, Kaleya and SCS1 in the nonsprayed plots. The seed weights for all cultivars from the benomyl-protected plots were significantly more than those from plots that were not sprayed with benomyl. SCS1 and Hernon-147 had the highest (27.7 and 27.5 %) reductions in seed weight while Magoye had a very negligible (1.2%) reduction in seed weight. The range of values of seed weights, were by comparison, not as great as yields based on percentages from benomyl-protected plots. Correlation analyses between disease severity, AUDPC with yield showed significant ($P \leq 0.01$) negative correlation coefficients of -0.77 and -0.73 respectively and 300-seed weight had a significant negative correlation coefficients ($P \leq 0.05$) of -0.73 and -0.63 for disease severity and AUDPC respectively.

Table 3: Disease severity, area under disease progress curve (AUDPC), yield from nonsprayed and benomyl-sprayed plots of soybean cultivars resistant and susceptible to frogeye leaf spot at Zamseed farm, 1997.

Cultivar, disease reaction[a]	Treatment No. of sprays	Severity [x]	AUDPC [y]	Yield kg ha^{-1}	Loss	Seed weight [z] g per 300	Loss
Hernon-147 (S)	0	47.65^{a*}	998.2 ^{b*}	1000^{e*}	35.6	34.2^{de*}	27.5
	2	11.58 ^{c}	245.0 ^{d}	1555 ^{cd}		47.2 ^{a}	
Magoye (R)	0	5.30 ^{c}	83.0 ^{f}	1444 ^{d}	26.7	40.6 ^{b}	1.2
	2	5.00 ^{c}	81.5 ^{f}	1972 ^{b}		41.4 ^{b}	
Kaleya (mR)	0	22.50 ^{b}	283.7 ^{c}	1157 ^{e}	30.5	35.8 ^{d}	5.0
	2	8.03 ^{c}	83.0 ^{f}	1666 ^{c}		37.7 ^{c}	
SCS1	0	50.23 ^{a}	1048.0^{a}	1456 ^{d}	37.2	34.2 ^{de}	27.7
	2	5.82 ^{c}	120.6^{e}	2320 ^{a}		47.3 ^{a}	
LSD		6.52	15.12	16.56		1.80	
CV (%)		14.74	2.54	5.87		2.73	

[a] S= susceptible; MR= moderately resistant and R= resistant ;[x] Severity expressed as the percentage of the total leaf are diseased at growth stage R1 to R4.

[y] Area under disease progress curve (AUDPC) determined by the formula presented by Shaner and Finney (1977)

[z] Percent yield and seed weight losses, based on comparisons within replications to sprayed plots.

4.4Characteristics of *Cercospora sojina* isolates

The isolates showed great variability in terms of conidial length. The mean conidial length did not differ significantly ($P \leq 0.05$) among isolates Cs-03, Cs-04, Cs-05 and Cs-06; the conidial length for isolates Cs-02, Cs-07 and Cs-09 was smaller and did not differ while isolates Cs-08 had the greatest conidial length followed by isolate Cs-01. There was a wide range in conidial lengths within and among the isolates. Isolate Cs-08 had the greatest length 69.3 μm while isolates Cs-03, Cs-04, Cs-05 and Cs-06 had the smallest lengths averaging 55.5 μm and isolates Cs-02 and Cs-09 had intermediate length of 60.0 μm (table 4).

The conidial width also varied considerably among the isolates. There was lack of variation in conidial width for isolates Cs-01, CS-02, Cs-03, Cs-04, Cs-07 and Cs-09. Conidial width of isolates Cs-05 and Cs-06 tended to be shorter. The lowest conidial width of 5.0 μm was observed in isolates Cs-06 (table 4).

Table 4: Measurements of conidial size of *Cercospora sojina* Hara isolates produced on water agar at 25°C [y]

	Length and width of conidia (μm)					
	Length			Width		
Isolate	Minimum	Maximum	Mean	Minimum	Maximum	Mean
Cs-01	47.5	100.0	65.2[b*]	4.4	9.0	7.4[a*]
Cs-02	45.0	80.0	60.5 [c]	4.5	9.0	7.6[a]
Cs-03	37.5	63.0	52.3 [d]	5.0	9.0	7.3[a]
Cs-04	35.0	63.0	52.7 [d]	4.0	7.0	5.3[c]
Cs-05	43.0	70.0	54.5 [d]	4.0	6.5	5.5[c]
Cs-06	43.0	70.0	55.0 [d]	4.4	6.5	5.0[c]
Cs-07	45.0	75.0	59.3 [c]	4.0	9.0	7.2[a]
Cs-08	57.0	82.0	69.3 [a]	5.0	8.5	6.6[b]
Cs-09	47.0	72.0	59.9 [c]	4.0	9.0	7.3[a]
Average	44.4	75.0	58.7	4.4	8.4	6.8
LSD	3.44				0.58	
CV (%)	13.32				19.63	

* Means followed by the same letter in each column were not significantly different at 0.05 probability level according to Duncan Multiple Range Test.

[y] Means of 40 observations from each replication.

Conidial shape fell within the reported categories: cylindrical with acute tips, cylindrical with obtuse tips, fusiform with acute tips and elongate with acute tips. Just like in the classification by length, isolates Cs-01, Cs-02, Cs-03, Cs-07, CS-08 and CS-09 fell within the same category while isolates Cs-04 and Cs-05 were in another category and isolate Cs-06 had a category of its own (table 5).

Table 5: Shape and number of septa in conidia of *Cercospora sojina* Hara (L.) isolates grown on water agar at 25°C [a]

	CONIDIA			
	Shape	Number of septa		
Isolates		Minimum	Maximum	Means
Cs-01	Cylindrical with obtuse tips	3	9	6^{bc} *
Cs-02	Cylindrical with obtuse tips	4	10	6^{bc}
Cs-03	Cylindrical with obtuse tips	3	9	5^{de}
Cs-04	Fusiform with acute tips	3	9	5^{de}
Cs-05	Fusiform with acute tips	3	8	5^{de}
Cs-06	Elongate with acute tips	3	7	5^{de}
Cs-07	Cylindrical with obtuse tips	3	8	5^{de}
Cs-08	Cylindrical with obtuse tips	4	10	7^{a}
Cs-09	Cylindrical with obtuse tips	4	8	6^{bc}
Average		3	7	6
LSD	0.585			
CV (%)	23.68			

* Means followed by the same letter were not significantly different at 0.05 probability level according to Duncan Multiple range Test.

[a] Means of 40 observations from each replication.

4.5 Colony growth of *Cercospora sojina* isolates

The colony diameter of *Cercospora sojina* differed on the second day of growth and interaction of media and isolates was significant . Isolates Cs-04, Cs-07, Cs-08 and Cs-09 had the highest growth rate with an average colony diameter of 3.2 millimetres; isolates Cs-02 and Cs-06 had the lowest diameter averaging 2.5 millimetres whereas isolates Cs-01, Cs-03 and Cs-05 did not differ significantly ($P \leq 0.05$) in their growth on Carrot leaf decoction agar (table 6). Growth rate of isolates on Potato dextrose agar and Sabouraud dextrose agar did not show any significant differences ($P \leq 0.05$) from the third to the ninth day.

On the sixth day of growth isolates Cs-06 and Cs-09 produced the largest colonies with a diameter of 6.1 millimetres on both CLDA and PDA. There were no significant differences in growth of colonies among the isolates on the sixth as well as the ninth day. Isolates Cs-01, Cs-02, Cs-03 and Cs-04 produced the smaller colonies (average diameter 5.4 millimetres) while isolates Cs-05 and Cs-07 had the smallest colony diameters averaging 4.5 millimetres (table 6).

On the ninth day of growth on CLDA, isolates Cs-02, Cs-06 and Cs-09 had the largest colony diameter averaging 8.4 millimetres followed by Cs-01, Cs-03, Cs-04 and Cs-08 which produced relatively smaller colonies averaging 7.7 millimetres. Isolates Cs-05 and Cs-07 developed smallest colonies averaging 6.6 millimetres. Colony diameters taken at the ninth day revealed that there were very little differences between colony growth on CLDA and PDA. On this day isolates Cs-02 and Cs-06 produced the largest colony diameter (8.8millimetres) and this was observed on PDA. Colony growth on Sabouraud dextrose agar was considerably

slower than on PDA and CLDA throughout the growth period. However, only a small increase in colony diameter was noted for all isolates on CLDA after the ninth day. The study reveals that CLDA serves as a better medium for growth of *Cercospora sojina* whereas PDA comes second.

Table 6: General growth pattern of *Cercospora sojina* Hara (L.) isolates (colony diameter in millimetres) on Carrot leaf decoction agar (CLDA), potato dextrose agar (PDA) and Sabouraud dextrose agar (SDA) in relation to time at 25^{0}C.

COLONY GROWTH [a]				
		After culturing for		
Medium	Isolate	3	6	9 days
CLDA	Cs-01	3.0 abcd*	5.6 abcd*	8.0 abcd*
	Cs-02	2.6 def	5.5 abcd	8.4 abc
	Cs-03	3.1 abc	5.5 abcd	7.8 abcde
	Cs-04	3.2 ab	4.9 abcde	7.2 abcdef
	CS-05	2.7 bcde	4.6 bcdef	6.7 bcdefg
	Cs-06	2.4 efg	6.1 ab	8.5 abc
	Cs-07	3.4 a	4.4 bcdef	6.4 cdefg
	CS-08	3.2 ab	5.7 abc	7.9 abcd
	Cs-09	3.4 a	6.1 ab	8.3 abc
Average		3.0	5.4	7.7
PDA	Cs-01	1.7 ijklm	3.5 ef	6.9 abcdef
	Cs-02	1.6 klm	5.4 abcd	8.7 ab
	Cs-03	1.9 hijk	5.0 abcde	7.6 abcde
	Cs-04	2.2 ghi	5.2 abcd	7.3 abcdef
	Cs-05	2.3 fgh	5.7 abc	7.8 abcde
	Cs-06	2.4 efg	6.5 a	8.9 a
	Cs-07	1.6 klm	5.0 abcde	7.2 abcdef
	Cs-08	1.8 hijklm	5.2 abcd	7.7 abcde
	Cs-09	2.7 cdef	6.1 ab	8.6 abc
Average		2.0	5.3	7.9
SDA	Cs-01	1.4 lm	3.5 ef	5.6 egf
	Cs-02	1.5 klm	5.4 abcd	6.6 bcdefg
	Cs-03	1.7 ijklm	5.0 abcde	5.9 defg
	Cs-04	2.1 ghij	5.2 abcd	7.8 abcde
	Cs-05	1.9 hijkl	3.3 f	5.1 fg

	Cs-06	1.9 hijkl	4.2 cdef	6.4 cdefg
	Cs-07	1.7 jklm	3.3 f	5.1 fg
	Cs-08	1.8 ijklm	4.8 cdef	6.0 defg
	Cs-09	1.4 m	3.0 f	4.7 g
Average		1.7	3.8	5.9
LSD		0.40	1.38	1.84
CV (%)		11.10	17.52	15.68

* Means followed by the same letter in each column were not significantly different at 0.05 probability level according to Duncan Multiple Range Test.

[a] Average of three replications

4.6 Colour of aerial mycelium in isolates of *Cercospora sojina.*

The aerial mycelium was white and changed to dark grey as the colonies grew older. This was observed in isolates Cs-03, Cs-04, Cs-05, Cs-06, Cs-07 and Cs-09 while isolates Cs-01, Cs-02 and Cs-08 changed from white with fine to cottony hyphae and raised colonies on PDA (table 7).

On SDA colour of aerial hyphae changed from whitish to light dark as they grew older. This was true for isolates Cs-01, Cs-05, Cs-06, Cs-07 and Cs-09. However isolates Cs-03 and Cs-04 had the aerial mycelium remaining whitish throughout the growth period while isolates Cs-02 and Cs-08 remained light dark throughout the culture period (table 8).

Colour change of the aerial mycelium on CLDA was variable among the isolates. The aerial mycelium changed from whitish to dark brown for isolates Cs-02, Cs-03, Cs-04, Cs-05, Cs-06, Cs-07 and from white to olive grey for isolates Cs-01, Cs-08 and Cs-09 (table 9).

Table 7: Colour of aerial hyphae of *Cercospora sojina* Hara (L.) isolates on potato dextrose agar at 25^0 C.

	After culturing for			
Isolate	3	6	9	12 days
Cs-01	whitish	cottony raised	cottony raised	cottony raised
Cs-02	whitish	cottony raised	cottony raised	cottony raised
Cs-03	whitish	whitish	dark grey	dark grey
Cs-04	whitish	whitish	dark grey	dark grey
Cs-05	whitish	whitish	dark grey	dark grey
Cs-06	whitish	whitish	dark grey	dark grey
Cs-07	whitish	whitish	dark grey	dark grey
Cs-08	whitish	cottony raised	cottony raised	cottony raised
Cs-09	whitish	whitish	dark grey	dark grey

Source of colony colour: Munsell soil colour charts (Appendix XIV)

Table 8: Colour of aerial hyphae of *Cercospora sojina* Hara (L.) isolates on Sabouraud dextrose agar at 25^{0}C.

	After culturing for			
Isolate	3	6	9	12 days
Cs-01	whitish	whitish	whitish	light dark
Cs-02	light dark	light dark	light dark	light dark
Cs-03	whitish	whitish	whitish	whitish
Cs-04	whitish	whitish	whitish	whitish
Cs-05	whitish	whitish	whitish	light dark
Cs-06	whitish	whitish	whitish	light dark
Cs-07	whitish	light dark	light dark	light dark
Cs-08	light dark	light dark	light dark	light dark
Cs-09	whitish	whitish	whitish	light dark

Source of colony colours: Munsell soil colour charts (Appendix XIV).

Table 9: Colour of aerial hyphae of *Cercospora sojina* Hara (L.) isolates on carrot leaf decoction agar at 25°C

	After culturing for			
Isolate	3	6	9	12 days
Cs-01	whitish	olive grey	olive grey	olive grey
Cs-02	whitish	brownish yellow	dark brown	dark brown
Cs-03	whitish	brownish yellow	dark brown	dark brown
Cs-04	whitish	brown	dark brown	dark brown
Cs-05	whitish	brown	dark brown	dark brown
Cs-06	whitish	reddish brown	dark brown	dark brown
Cs-07	whitish	reddish brown	dark brown	dark brown
Cs-08	whitish	olive grey	olive grey	olive grey
Cs-09	whitish	olive grey	olive grey	olive grey

Source of colony colours: Munsell soil colour charts (Appendix XIV)

4.7 Colour of submerged mycelium in isolates of *Cercospora sojina.*

The colour of submerged hyphae in all isolates showed differences on the three media as shown in tables 10, 11 and 12. Because the range of colours among the isolates overlapped, colour of colony of the isolates could not be used as a means of separation.

Table 10: Colour of submerged hyphae of *Cercospora sojina* Hara (L.) isolates on potato dextrose agar at 25^{0}C.

		After culturing for			
Isolate	Side	3	6	9	12 days
Cs-01	Top	olive grey	olive grey	olive grey	olive grey
	Reverse	dark brown	dark brown	dark brown	dark brown
CS-02	Top	olive grey	olive grey	olive grey	olive grey
	Reverse	dark brown	dark brown	dark brown	dark brown
Cs-03	Top	olive grey	dark brown	dark brown	dark brown
	Reverse	light dark	light dark	light dark	dark brown
Cs-04	Top	greyish	greyish	greyish	greyish
	Reverse	dark brown	dark brown	dark brown	dark brown
Cs-05	Top	whitish	whitish	greyish	greyish
	Reverse	dark brown	dark brown	dark brown	dark brown
Cs-06	Top	whitish	whitish	greyish	greyish
	Reverse	light dark	dark brown	dark brown	dark brown
Cs-07	Top	whitish	whitish	olive grey	olive grey
	Reverse	light dark	dark brown	dark brown	dark brown
Cs-08	Top	whitish	whitish	olive grey	olive grey
	Reverse	dark brown	dark brown	dark brown	dark brown
Cs-09	Top	whitish	whitish	greyish	greyish
	Reverse	light dark	dark brown	dark brown	dark brown

Source of colony colours: Munsell soil colour charts (Appendix XIV)

Table 11: Colour of submerged hphae of *Cercospora sojina* Hara (L.) isolates on Carrot leaf decoction agar at 25°C.

		After culturing for			
Isolate	Side	3	6	9	12 days
Cs-01	Top	whitish	dark brown	dark brown	dark brown
	Reverse	whitish	olive grey	olive grey	olive grey
Cs-02	Top	brownish yellow	brownish yellow	dark brown	dark brown
	Reverse	brownish yellow	brownish yellow	dark brown	dark brown
Cs-03	Top	whitish	dark brown	dark brown	dark brown
	Reverse	brownish yellow	brownish yellow	dark brown	dark brown
Cs-04	Top	olive grey brown	olive grey	olive grey	olive grey
	Reverse	brown	brown	dark brown	dark brown
Cs-05	Top	grey	grey	grey	grey
	Reverse	brown	yellowish brown	dark brown	dark brown
Cs-06	Top	olive grey	olive grey	olive grey	olive grey
	Reverse	brown	reddish brown	dark brown	dark brown
Cs-07	Top	dark brown	dark brown	dark brown	dark brown
	Reverse	olive grey	olive grey	olive grey	olive grey
Cs-08	Top	whitish	yellowish brown	dark brown	dark brown o
	Reverse	whitish	olive grey	olive grey	olive grey
Cs-09	Top	whitish	reddish brown	dark brown	dark brown
	Reverse	brown	reddish brown	dark brown	dark brown

Source of colony colours: Munsell soil colour charts (Appendix XIV).

Table 12: Colour of submerged hyphae of *Cercospora sojina* Hara (L.) isolates on sabouraud dextrose agar at 25°C.

		After culturing for			
Isolate	Side	3	6	9	12 days
Cs-01	Top	whitish	whitish	greyish	greyish
	Reverse	whitish	whitish	dark brown	dark brown
Cs-02	Top	whitish	whitish	greyish	greyish
	Reverse	whitish	whitish	dark brown	dark brown
Cs-03	Top	whitish	whitish	whitish	whitish
	Reverse	whitish	whitish	dark brown	dark brown
Cs-04	Top	whitish	whitish	whitish	whitish
	Reverse	whitish	whitish	dark brown	dark brown
Cs-05	Top	whitish	whitish	whitish	greyish
	Reverse	whitish	dark brown	dark brown	dark brown
Cs-06	Top	whitish	olive grey	olive grey	olive grey
	Reverse	dark brown	dark brown	dark brown	dark brown
Cs-07	Top	whitish	whitish	greyish	greyish
	Reverse	dark brown	dark brown	dark brown	dark brown
Cs-08	Top	whitish	olive grey	olive grey	olive grey
	Reverse	dark brown	dark brown	dark brown	dark brown
Cs-09	Top	whitish	olive grey	olive grey	olive grey
	Reverse	whitish	dark brown	dark brown	dark brown

Source of colony colours: Munsell soil colour charts (Appendix XIV)

4.8 Colony margin in isolates of *Cercospora sojina* on three different media.

Differences among the isolates in the shapes of the colony margins on the three media were noticed. The results are described in Table 13.

Table 13: Colony margin in isolates of *Cercospora sojina* Hara (L.) after nine days of growth on Potato dextrose agar, Sabouraud dextrose agar Carrot leaf decoction agar at 25°C [a]

	PDA	SDA	CLDA
Isolate	Margin type	Margin type	Margin type
Cs-01	Sinuate	Sinuate	Sinuate
Cs-02	Sinuate	Sinuate	Prostrate
Cs-03	Entire	Prostrate	Prostrate
Cs-04	Entire	Prostrate	Prostrate
Cs-05	Entire	Prostrate	Prostrate
Cs-06	Entire	Prostrate	Prostrate
Cs-07	Entire	Prostrate	Prostrate
Cs-08	Sinuate	Sinuate	Sinuate
Cs-09	Sinuate	Sinuate	Sinuate

[a] Colony margin based on Stalpers' descriptions (1978).

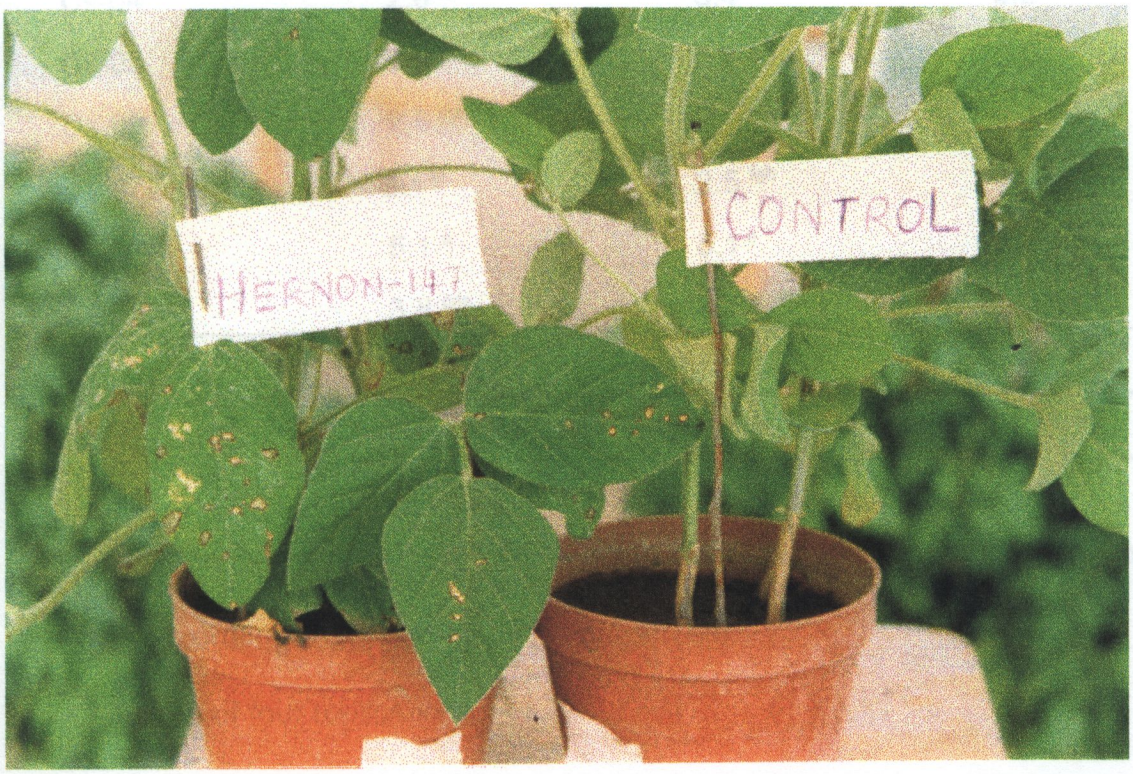
4.9 Pathogenicity and virulence of isolates of *Cercospora sojina*

All the nine isolates of *Cercospora sojina* were pathogenic to soybeans. Of the four cultivars Hernon-147, SCS1, Kaleya and Magoye used in testing pathogenicity only Magoye was resistant to all the isolates as it did not differ with the controls.

First frogeye leaf spot symptoms appeared 10, 12, 14, and 15 days after inoculation and the greenhouse temperature ranged from 23°C to 30°C. The disease developed most rapidly and incited most severe symptoms at 30°C. Examining table 14 it is noticed that *Cercospora sojina* isolates varied greatly in virulence to the various soybean cultivars on which they were tested. There was a significant cultivar X isolate interaction. Lesion diameter varied significantly from a mean of 2.0 millimetres caused by isolates Cs-03, Cs-07 on Kaleya to 5.1 millimetres incited by isolates Cs-02 and Cs-08 on Hernon-147 and SCS1 cultivars. Thus, isolates Cs-02 and Cs-08 produced the largest lesion size (5.1 millimetres) on Hernon-147, SCS1 and Kaleya, this was followed by isolate Cs-01 which produced lesions of 4.8 millimetres on SCS1, 4.3 millimetres on Kaleya and 4.1 millimetres on Hernon-147 (table14). Isolates Cs-04, Cs-05 and Cs-06 were among the least virulent on all the three cultivars. Differences in resistances and susceptibility of cultivars to all isolates were evident in the size of the lesions. The cultivar Kaleya showed some degree of resistance against the isolates especially isolates Cs-03,-Cs-04, Cs-05, Cs-06 and Cs-07. Hernon-147 and SCS1 were the most susceptible cultivars to most isolates.

Figure 6: Symptoms of Frogeye leaf spot on test cultivars 14 days after inoculation.


SCS1
KALEYA


HERNON-147
CONTROL

5.0 DISCUSSION

In the field, frogeye leaf spot symptoms were found most frequently as from the R2 stage of soybean growth. Results of this study are all from tests that relied on natural infection for the establishment of disease. The present study, therefore had several advantages (a) the disease develops from naturally occurring inoculum; (b) the inoculum represents the range of strains of the pathogen occurring at each location and is not limited to a single strain selected by the investigator; (c) the strains are naturally aggressive, neither attenuated by being maintained in culture nor selected for exceptional virulence; and (d) infections occur continuously over a period of time, not by administration of arbitrary doses of inoculum at discrete times to the crops in the field.

Incidence of frogeye leaf spot of soybeans was lower in Central province as compared to Lusaka and Southern provinces. The higher incidence in Lusaka and Southern provinces could be attributed to differences in environmental factors for example the higher rainfall amount these two provinces received over the three months the survey was carried out. Heavy rains and the high temperatures during the season (Appendices II and III) caused higher incidence as opposed to Central province which had low rainfall over the three months because of the dry and hot weather not favourable for the development of the disease. Frogeye leaf spot of soybeans is favoured by warm, humid weather (Sinclair and Backman, 1989). Mean temperatures for the three months were 27.1°C for Central province, 26.5°C and 28.1°C for Lusaka and Southern provinces respectively (appendix III). Total rainfall for the three months were 472.4 millimetres for Central province, 572.8 and 659.0 millimetres for Lusaka and Southern provinces respectively.

The low severity of frogeye leaf spot in Central province could also be accounted to the differences in cropping practices. Interviews with soybean farmers in Central province revealed less cultivation of the crop in the province. Furthermore, most farmers rotated their soybean crop with wheat and therefore the conditions were not favourable for the increase in the inoculum. The farming history of most of the farms in Lusaka and Southern provinces recorded intensive cultivation of soybean every year. The potential of the pathogen to initiate a disease year after year is greatly influenced by its capacity to overwinter (Sinclair and Backman,1979). Apparently continuous cultivation of the crop in Lusaka and Southern provinces made *Cercospora sojina* survive without difficulty since it can be recovered easily from the infected debris.

Low incidence and severity of the disease in Central province could also be due to less virulent races of *Cercospora sojina* as supported by the level of virulence of the isolates obtained from this province. Isolates Cs-04, Cs-05 and Cs-06 produced very small lesions on all cultivars in the greenhouse. These small lesions or flecks could easily be distinguished from the large spreading lesions caused by isolates Cs-01, Cs-02 obtained from Lusaka and isolate Cs-08 from Southern province. Disease severity was greatest on plants that did not receive any benomyl treatment. The severity was very high for Hernon-147 (45.64 %) and SCS1 (45.46 %).

Benomyl applications controlled frogeye leaf spot by slowing the rate of disease development. Seed yield of all sprayed cultivars was significantly greater than that of the same nonsprayed cultivars. SCS1 had the greatest yield reduction compared with the benomyl sprayed control (37.2 %) followed by Hernon-147(35.6 %), Kaleya (30.5 %) and Magoye (26.7 %). These effects may be attributed to reduced photosynthesis due to reduced available photosynthetic area on the leaves. This in turn leads to

reduced movement of metabolites to developing seeds with seed size reduction as the normal consequence. The reductions in seed weights were more pronounced on nonsprayed SCS1 (27.7 %) and Hernon-147 (27.5 %), therefore reduction in seed weight was shown to be a primary component of yield loss. This has also been reported by Hartman, *et al.,* (1987). These results are also in agreement with results of Kamicker and Lim (1985) who reported that foliage, stems and pods of benomyl-treated plants always remain green longer than the control and the delay in harvest, induced by the application of fungicide, could be contributory to the yield increases by providing a few extra days for seeds to fill.

Decrease in yield due to reduction in seed weight for nonsprayed cultivars indicate that increases in yield may serve as an indicator of pathogen activity because increases or decreases in yields generally correspond to the activity of the fungicides or the pathogens involved. For yield reduction in nonsprayed plots one explanation could be that yield reduction occured from damaged to plants which were severely infected. For example, infection of the peduncle before the seeds have had a chance to fill completely could result in the seeds not acquiring their maximum weight.

The significantly higher yield reduction in Magoye (26.7 %) for the unprotected plots was due to attack by Red leaf blotch or Pyrenochaeta leaf blotch. Occurrence of this disease was very high only on unprotected plots of the cultivar Magoye and it caused a higher rate of defoliation. Prevalence of Pyrenochaeta leaf blotch on the other three cultivars was very insignificant to bring about any yield losses.

Considerable morphological and physiological variation of the nine isolates was noted on the basis of size of conidia, shape, colony colour on CLDA, PDA and SDA, growth

rate in culture and virulence of the isolates. The size of conidia varied and the measurements agree with those reported by Chupp (1953). Variation in length of conidia within isolates was greater than the variation among isolates. On the basis of length of conidia alone the isolates fell into four groups; isolate Cs-08 averaging the longest (69.3 μm) followed by isolate Cs-01 (65.2 μm) and isolates Cs-02, Cs-09, and Cs-07 which averaged 60.5, 59.9 and 59.3 μm respectively. Isolates Cs-03, Cs-04, Cs-05, and Cs-06 averaged the shortest (53.6 μm).

On the basis of conidial width, which is considered important in race classification of *Cercospora sojina*, the isolates were grouped into three classes . Isolates Cs-01, Cs-02, Cs-03, CS-07 and Cs-09 had the greatest width averaging 7.4μ m. Isolate Cs-08, with conidial width of 6.6μm, belonged to its own group while Cs-04, Cs-05 and Cs-06 had the least conidial width averaging 5.3 μm. Conidia of isolates in the first group averaged 1.0-2.0 μm wider than those of the last group therefore isolates Cs-01, Cs-02, Cs-03, Cs-07 and Cs-09 could have belonged to race II while isolates Cs-04, Cs-05 and Cs-06 could be *Cercospora sojina* race I. These results resemble those of Chupp (1953) who reported an average of 1.0-1.5 μm conidia of race II being wider than those of race I. The differences among isolates of *Cercospora sojina* signify their inherent variability.

Conidial shape fell within the reported categories; cylindrical with obtuse tip, fusiform with acute tips and elongate with acute tips. Likewise isolates Cs-01, Cs-02, Cs-03, Cs-07, Cs-08 and Cs-09 fell within the same category while Cs-04, Cs-05 fell in another category. Isolate Cs-06 had a class of its own. This variation in shapes seem to be an inherent feature of isolates of *Cercospora sojina*. The evidence presented in this study supports the contention of Chupp (1953) that only a few basal types are distinct enough

to make unfailing classes among species of *Cercospora sojina*.

Different growth rates among isolates of *Cercospora sojina* again appear to be influenced by their inherent characters. Growth rate was highest in isolates Cs-02, Cs-06 and Cs-09 on CLDA on the third and sixth day, there was very little difference on PDA and CLDA on the ninth day. There was constantly poor growth of isolates on SDA. Isolates with high growth rates might have used nutrients in the medium more efficiently than those with the poor growth rate. One notable characteristic illustrated is that medium prepared from tissues containing chlorophyll (CLDA) supported vigorous growth of the isolates up to the ninth day after which the growth rate slowed down. The nutritive quality were apparently more dominant when chlorophyll was present than when it was absent. These results agree in part with those reported by Kilpartick and Johnson (1956) who reported heavy sporulation and abundant mycelial growth of *Cercospora species* on CLDA steamed without pressure. They reported chemical change of substance in carrot leaves by pressure sterilization hence this retarded sporulation and mycelial growth. Slow growth rate of isolates after the ninth day could be due to earlier exhaustion of the nutrients in the medium.

Differences again existed among isolates in the margin shape which was either sinuate, prostrate or entire (Stalpers, 1978). Sinuate margin was observed in 44 percent of the isolates whereas entire margin was found in 56 percent on PDA and CLDA. These percentages were also true for the margin types on SDA. The variation in margin types among isolates could also be associated with the differences in growth rates. Isolates having higher growth rates developed sinuate type of margin while those with poor growth rate produced prostrate or entire margins. The ability of the isolates to differ in the contour of the margin again, demonstrates their variability.

In pathogenicity tests symptoms developed rapidly and the pathogen incited bigger lesion sizes when the temperature was above at 26°C. These results suggest that the temperature, after the plants had become infected and removed from the moisture chambers, had a greater effect on disease development than the temperature during the 7-day period immediately following inoculation. The relative virulence of isolates as defined by the different size of lesions signify variability in the pathogen. A striking difference was also measured between isolates from the same area. Isolates Cs-08 and Cs-09 were recovered from Mazabuka district from soybean fields less than one kilometer apart. Besides differences in virulence of the pathogen at different locations and differences in environmental conditions that may influence disease development, results of this research indicate that severity of the disease is also affected by the resistance of the cultivar. The resistant cultivars may be very important in future breeding programmes for frogeye leaf spot resistance. The significant main effects due to cultivar and to isolate, and the highly significant cultivar X isolate interaction suggest that both vertical and horizontal resistance to *C. sojina* occur in the soybean host lines and that races may exist among the isolates used in this study.

6.0 CONCLUSION

The high incidence of frogeye leaf spot on soybean from R1 growth stages suggested that the pathogen, *Cercospora sojina* was present early in the growing season. *Cercospora sojina* was recovered from four soybean cultivars namely SCS1, Hernon-147, Kaleya and Gazelle. This indicates the relative abundance of this fungus on a wide range of cultivars. The incidence of frogeye leaf spot on soybean measured in this study can largely be attributed to environmental factors and presence of ample and virulent inoculum. The high severity of 50.23% and yield reduction of 37.20% on SCS1 probably represents the upper limit of yield reduction attributable to frogeye leaf spot, whereas the mean yield reduction of 33.1% for the other two susceptible cultivars more nearly represent the reduction that might ordinarily be expected in a susceptible cultivar in agroecological zone II of Zambia. The study also indicates that frogeye leaf spot can be reduced by benomyl application that includes two sprays one at R1 and the other at R3. Reduction of frogeye leaf spot during early and midreproductive stages may increase yields when frogeye leaf spot would otherwise be severe and when the possibility of potential yields is high.

The highly significant negative correlation between the severity of the disease and grain yield of SCS1 and Hernon-147 indicate that the variations in grain yield were attributable to the effects of the disease on the cultivars. Average changes in seed yields calculated from the data indicate that losses due frogeye leaf spot usually become of increasing significance when the disease severity approached 40 %.

Isolates from the different locations and cultivars exhibited variability in morphology,

physiology and virulence. For example differences were noted between isolates from Lusaka and Central provinces. Such differences can best be recognised at the race level and should not be used to separate isolates that are morphologically indistinguishable. Symptoms, although influenced by the isolate, are largely determined by the cultivar and the environment. They are of little value for differentiating races of *Cercospora sojina* on soybeans. It should be suggested that inoculum of *C. sojina* for greenhouse and field inoculation be grown on carrot leaf decoction agar or Potato dextrose agar under alternating 12hr of light and dark at 25°C.

7.0 RECOMMENDATION

The relationship between severity of frogeye leaf spot and yield losses should further be investigated by continuing the study of the disease severity, area under disease progress curve and defoliation on yield components of soybeans. These studies will provide useful information for developing control measures for frogeye leaf spot through fungicide sprays and disease resistance. The high severity and wide distribution of frogeye leaf spot of soybeans in agroecological region II of Zambia should stimulate further monitoring of the incidence and severity of the disease in other agroecological zones. These studies should be done in conjunction with yield losses to facilitate prediction of potential damage to different soybean cultivars.

The extreme morphological and virulent variability among isolates of *Cercospora sojina* should be investigated further with collections of isolates from a wide range of geographical regions and soybean cultivars representing various genetic backgrounds. Any variety for release should be tested for resistance to frogeye and if found to be resistant then only they should be released.

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APPENDICES

Appendix I :Stage of development descriptions for soybeans, *Glycine max* (L.) Merril.

This system stage development descriptions apply to single plants or a community of plants and it is useful in all types of soybean research. The system has separate descriptions for vegetative and reproductive development relationships that can occur in sawbones. Vegetative stage numbers are preceded by V and reproductive stages by R. Plants can be described by for both vegetative and reproductive development if desired.

Vegetative stages

Vegetative stages are determined by counting the number of nodes on the main stem, beginning with the unifoliolate node, which have or have had a completely unrolled leaf. A leaf is considered completely unrolled when the leaf at the node immediately above it has unrolled sufficiently so the edges of each leaflet are no longer touching. At the terminal node on the main stem, the leaf is considered completely unrolled when the leaflets are flat and similar in appearance to older leaves on the plant.

Stage no.	Descriptions
V1	Completely unrolled leaf at the unifoliolate node.
V2	Completely unrolled leaf at the first node above the unifoliolate node.
V3	three nodes on the main stem beginning with the unifoliolate node.
V(N)	N nodes on the main stem beginning with the unifoliolate node.

Reproductive stages

Stage No.	Description
R1	One flower at any node.
R2	Flower at node immediately below the uppermost node with a completely unrolled leaf.
R3	Pod 0.5 cm(¼ inch) long at one of the four uppermost nodes with a completely unrolled leaf.
R4	Pod 2 cm (¾ inch) long at one of the four uppermost nodes with a completely unrolled leaf.
R5	Beans beginning to develop (can be felt when the pod is squeezed) at one of the four uppermost nodes with a completely unrolled leaf.
R6	Pod containing full size green beans at one of the four uppermost nodes with a completely unrolled leaf.
R7	Pods yellowing; 50% of leaves yellow. Physiological maturity.
R8	95% of pods brown. Harvest maturity.

Appendix II: Monthly rainfall totals (in mm) for 1996/1997 growing season.

Station	Year	Month	Value	Valid days	Dry dekads (≤30 mm)
Kabwe	1996	October	0.0	31	3
	1996	November	115.9	30	1
	1996	December	356.1	31	0
	1997	January	196.9	31	0
	1997	February	176.2	28	1
	1997	March	99.3	31	2
	1997	April	0.0	30	3
Lusaka	1996	October	0.0	31	3
	1996	November	77.8	30	1
	1996	December	148.7	31	1
	1997	January	296.9	31	0
	1997	February	169.2	28	1
	1997	March	106.7	31	3
	1997	April	25.7	30	2
Mazabuka	1996	December	165.4	31	1
	1997	January	361.9	31	0
	1997	February	169.6	28	1
	1997	March	127.5	31	2
	1997	April	71.5	30	1

Appendix III :Maximum and Minimum temperatures (°C) recorded from three stations during the 1996/1997 season.

Station	Year	Month	Maximum	Minimum	Valid days
Kabwe	1996	October	32.8	18.2	31
	1996	November	31.5	19.2	30
	1996	December	27.3	17.8	31
	1997	January	26.8	18.0	31
	1997	February	26.4	17.4	28
	1997	March	28.1	17.4	31
	1997	April	25.5	11.0	31
Lusaka	1996	October	32.1	18.7	31
	1996	November	30.3	18.8	30
	1996	December	26.7	17.9	31
	1997	January	26.2	17.7	30
	1997	February	25.5	17.0	28
	1997	March	27.7	17.4	30
	1997	April	26.2	14.5	30
Mazabuka	1996	October	35.1	17.8	31
	1996	November	32.6	19.4	30
	1996	December	28.5	18.7	31
	1997	January	28.1	19.3	31
	1997	February	27.4	18.3	28
	1997	March	28.7	17.9	31
	1997	April	27.6	14.8	30

Appendix IV: Temperatures in the greenhouse where the plants were grown at the time and after inoculation in ° C.

Date	Maximum	Minimum
7.7.97	19	17
8.7.97	19	12
9.7.97	18	14
10.7.97	23	14
11.7.97	18	13
12.7.97	18	13
13.7.97	18	12
14.7.97	23	13
15.7.97	25	13
16.7.97	26	13
17.7.97	30	16
18.7.97	26	13
19.7.97	25	15
20.7.97	23	13
21.7.97	22	13
22.7.97	24	14
23.7.97	27	12
24.7.97	29	14
25.7.97	29	16
26.7.97	30	16
27.7.97	29	16
28.7.97	30	17
29.7.97	29	16
30.7.97	29	16
31.7.97	27	14
1.8.97	28	16
2.8.97	27	16

Appendix V: Analysis of Variance for disease incidence ratings for the three provinces and four soybean cultivars.

Source of variation	Degree of freedom	Sum of Squares	Mean square	F Value
Replication	2	0.91	0.46	4.45
Cultivars	3	59.66	19.89	194.52*
Provinces	2	18.62	9.31	91.07*
Cultivar X provinces	6	12.26	2.04	19.99*
Error	22	2.25	0.10	
Total	35	93.70		
CV (%) 13.83				

* Significantly different at 0.05 probability level.

Appendix VI: Analysis of variance for area under disease progress curve for the three provinces and four soybean cultivars.

Source of variation	Degree of freedom	Sum of squares	Mean square	F value
Replication	2	44133.70	22066.85	5.90
Cultivars	3	3123344.30	1041114.77	278.55*
Provinces	2	835284.62	417642.31	11.74*
Cultivar X Prov.	6	616787.66	101797.94	27.24*
Error	22	82227.56		
Total	35	4695777.84		
CV (%)	12.79			

* Significantly different at 0.05 probability level.

Appendix VII: Analysis of Variance for area under disease progress curve for benomyl-sprayed and sprayed four soybean cultivars [y].

Source of variation	Degree of freedom	Sum of Squares	Mean square	F value
Replication	2	397.05	198.53	1.44
Cultivar	3	708442.65	354221.33	2565.33*
Sprays	1	1769243.51	1769243.51	12813.18*
Cultivar X Spray	2	431689.23	215844.61	1563.18*
Error	10	1380.80	138.08	
Total	18	2911153.24		
CV (%) 2.54				

* Significantly different at 0.05 probability level.

Appendix VIII: Analysis of variance for severity of frogeye leaf spot for benomyl-sprayed and nonsprayed cultivars.

Source of variation	Degree of freedom	Sum of squares	Mean square	F value
Replication	2	1.27	0.63	0.05
Cultivar	3	742.28	371.14	28.93*
Sprays	1	4507.75	4507.75	351.43*
Cultivar X spray	2	716.56	358.28	27.93*
Error	10	128.27	12.83	
Total	18	6096.13		
CV (%) 14.74				

* Significantly different at 0.01 probability level.

Appendix IX: Analysis of variance for soybean yield in relation to treatment by benomyl.

Source of variation	Degree of freedom	Sum of squares	Mean square	F value
Replication	2	10728.09	5364.05	0.63
Cultivars	3	1384988.68	461662.89	54.24*
Sprays	1	226098.77	2262098.77	265.75*
Cultivar X spray	3	126150.84	42050.28	4.94*
Error	14	119167.75	8511.98	
Total	23	1867134.13		
CV (%) 5.87				

* Significantly different at 0.01 probability level

Appendix X: Analysis of variance for 300-seedweight of benomyl-sprayed and nonsprayed soybean cultivars.

Source of variation	Degree of freedom	Sum of squares	Mean square	F value
Replication	2	0.21	0.10	0.09
Cultivar	4	242.17	60.54	54.82*
Sprays	1	254.04	254.04	230.04*
Cultivar x sprays	4	264.77	66.19	59.94*
Error	18	19.88	1.10	
Total	29	781.07		
CV (%) 2.73				

* Significantly different at 0.05 probability level.

Appendix XI: Analysis of variance for conidial length of *Cercospora sojina* isolates.

Source of variation	Degree of freedom	Sum of Squares	Mean Square	F value
Replication	39	2447.98	62.77	1.03
Isolates	8	10721.15	1340.13	21.89 *
Error	312	19095.43	61.21	
Total	359	32265.06		
CV (%) 13.32)				

* Significantly different at 0.05 probability level.

Appendix XII: Analysis of variance for conidial width (in μm) of nine isolates of *Cercospora sojina.*

Source of variation	Degree of freedom	Sum of squares	*Mean square*	F value
Replication	*39*	86.72	*2.22*	1.26
Isolates	*8*	278.71	34.84	19.71*
Error	*312*	*551.46*	1.77	
Total	359	916.90		
CV (%) 19.63				

* Significantly different at 0.05 probability level.

Appendix XIII: Analysis of variance for soybean cultivars and *Cercospora sojina* virulence.

Source of variation	Degree of freedom	Sum of squares	Mean square	F value
Replication	3	1.34	0.45	1.59
Cultivar	2	12.26	6.13	21.83*
Isolates	8	111.13	13.89	49.46*
Cultivar X Isolate	16	9.28	0.58	2.06**
Error	78	21.91	0.28	
Total	107	155.92		
CV (%) 15.26				

* Significantly different at 0.05 probability level.

**Significantly different at 0.01 probability level.