EPIDEMIOLOGY AND MANAGEMENT OF GREY LEAF SPOT : A NEW DISEASE OF MAIZE IN SOUTH AFRICA

by

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Early lesions: pin-point spots and young elongated lesions

Characteristic mature lesions symptomatic of grey leaf spot (GLS) disease

Mature lesions which are greyish in colour due to sporulation

Lower leaves are usually the site for initial infection

GLS predisposes maize to stalk rotting and severe lodging (foreground) compared with fungicide treated maize (background)

◄ GLS reduced maize ear size and yield (centre) compared with ears from fungicide treated plants

Certain fungicides control GLS effectively. Compare fungicide treated healthy maize (left) and unsprayed diseased maize (right)

FRONTISPIECE : GREY LEAF SPOT IN MAIZE

ABSTRACT

Grey leaf spot is a relatively new fungal disease of maize in South Africa. It has become well established in the province of KwaZulu-Natal, and is capable of reducing grain yields by 20 to 60%. The disease is spreading to neighbouring provinces and countries. This study was conducted to establish solutions to the problem that could be easily implemented by maize farmers. Available literature was reviewed to establish the most appropriate epidemiologically based control measures that might be applicable in South Africa. Field trials were conducted to determine the effects of stubble and conventional tillage practices, cultivar susceptibility, fungicides, the correct time and frequency of fungicide treatment. and the financial benefits of fungicide treatment on grey leaf spot severity. The trials were evaluated for disease severity and grain yields.

No commercial hybrids were identified to be resistant to grey leaf spot in the maize hybrid response to grey leaf spot trial. However, subsets of high-yielding hybrids less-susceptible to disease were identified - including PAN 6480, CRN 3584, SNK 2154 and PAN 6578. The most susceptible hybrids were identified to include RS 5206, PAN 6552, A 1849, PAN 6528 and PAN 6140. Fungicides containing carbendazim/flusilazole, were found to be most effective in controlling disease and increased maize yields. Hybrids such as RS 5206 and RS 5232 highly susceptible to disease and showed the highest grain yield response to fungicide treatment, whilst least-susceptible hybrids, such as PAN 6480, had the lowest response. The tillage trial aimed at management practices to reduce grey leaf spot indicated fungicides to be more effective in managing disease than tillage practices aimed at a reduction of initial inoculum. Trials on chemical control of grey leaf spot identified fungicides of the triazole and benzimidazole chemical groups to be effective in controlling disease, but only combination products of these chemical groups, were registered, in support of the pathogen resistance strategy. Products registered were carbendazim/flusilazole, carbendazim/flutriafol and carbendazim/difenoconazole. The frequency and timing of fungicide applications for the control of grey leaf spot in maize studies identified spray treatments initiated when disease had progressed to the basal five leaves and, before the exponential phase of the epidemic, provided the most effective disease control and concomitant high grain yields. Further spray treatments were necessary with early disease infections, in order to provide disease control until crop physiological maturity. The final study on the economic benefits of fungicide treatment of grey leaf spot in maize in KwaZulu-Natal indicated that the highest added yield response was not necessarily the best parameter to justify fungicide treatment. Rather, the expected added profit was a better parameter. In this study the highest added profits were R1 400 ha^{-1} from the triple-spray programme in 1993/94 and R439 ha^{-1} from a single-spray in 1992/93. The optimum treatment choice depended on the individual's risk-return preferences, which reflect his level of risk-aversion.

An integrated approach using tillage practices, crop rotations, hybrids less-susceptible to the pathogen and the judicious use of fungicides is likely to be the most successful in controlling the disease. In the long term, the cornerstone of the integrated approach will be the development and use of hybrids resistant to the disease.

PREFACE

I hereby declare that the experimental work in this thesis was the result of my own investigation, under the supervision of Professor A.L.P. Cairns in the Department of Agronomy, Professor F.H.J. Rijkenberg, Dean, Faculty of Agriculture, and Mr. M.D. Laing in the Department of Microbiology and Plant Pathology, University of Natal, Pietermaritzburg, from March 1991 to January 1996.

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Dr G.A. Payne of North Carolina State University
Dr Walker Kirby of University of Illinois
Dr D.G. White, University of Illinois
I would especially like to thank Dr. C Martinson and Dr. F. Nutter Jr. of Iowa State University and Dr. E.L. Stromberg of Virginia Polytech and State University for their close co-operation and advice on grey leaf spot.
Finally, I would like to thank my colleagues at Cedara for their general encouragement throughout the project, and to Mrs. H. Mostert and Mrs. H. Morgan, for their patience in typing the papers in this thesis.

To my wife, Liz,
and my family,
go special thanks
for their support and
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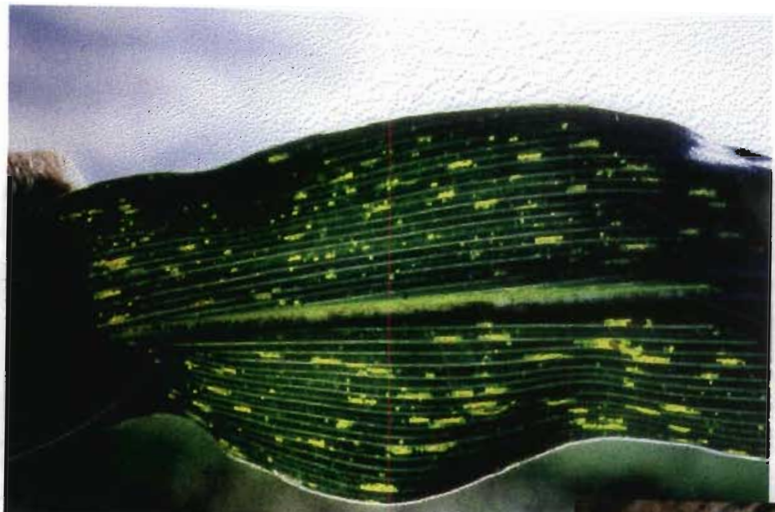
FRONTISPIECE

Early *Stenocarpella* ear rot infection.

Ear rot caused by *Stenocarpella* species.



Initial grey leaf spot infection..

Grey leaf spot photographs -
Ward, Birch and Nowell, 1993.

Blighting caused by Grey Leaf Spot.

CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

1.1 *INTRODUCTION*

Maize (*Zea mays* L.) is one of the most important agricultural crops grown in South Africa, accounting for 3,9 million ha, or over a third, of all crops cultivated in the country. In 1993/94 it had a marketable value of R4,13 billion (Anon., 1995a). The crop forms the staple diet of the majority of the South African population and is the main component in livestock rations. In 1993/94, 2,75 million tons of maize were sold for human consumption, 6,47 million tons were used as feed in the livestock industry and nearly 3 million tons were exported (Anon., 1995a). Not only is maize important from a production point of view, but the industry is also a large employer of labour.

In recent years there have been far-reaching changes in the maize industry. The producer prices are no longer determined on the basis of production costs, but on market-related factors. Producer prices vary according to market-demand and supply, and, in recent years, high costs of production have lowered profit margins. The effect of the combination of these factors has increased the risk of producing maize. To minimise this risk, input costs have to be minimised. Emphasis will in future be on high yields and on the most cost effective use of production inputs, such as disease and pest control, fertilizer usage and tillage practices (Schabort, 1990).

Pests and diseases cause significant crop losses, but the extent of these has not been quantified in South Africa (Chambers, 1986). Gevers, *et al.*, (1990), indicated that foliar and ear-rot epidemics resulted in considerable yield losses in the 1980s, but these diseases have since been overcome by the breeding of resistant hybrids. Grey leaf spot (GLS) disease, caused by the fungus *Cercospora zeae-maydis*. Tehon and

and E.Y. Daniels, is a more recent foliar disease of maize in South Africa. It was described by an eminent visiting American epidemiologist as having the potential to cause severe economic hardship to the maize industry in South Africa, because the disease occurs early in the host growth stage and at very high levels ([1]Nutter, pers. comm., 1994). The disease has the capability to reduce grain yields by as much as 60% and has become well established in maize growing areas in the province of KwaZulu-Natal. It has now been observed in neighbouring provinces (Ward & Nowell, 1997).

The increase in incidence and severity of GLS in the United States has been associated with continuous maize production and an increase in conservation tillage practices (Anderson, 1995; Stromberg & Donahue, 1986). Research workers in the United States recognise the value of stubble tillage in soil conservation and in sustainable farming (Latterell & Rossi, 1983; Stromberg, 1986). They believe the long-term solution to the problem is genetic resistance, which is a highly efficient and cost-effective method of control (Lipps & Pratt, 1989). Because of the large annual losses of soil due to erosion and the advantages of crop residues on the soil surface in conserving soil and soil moisture, stubble tillage should be more encouraged in South Africa, as it plays an important role in sustainable agriculture. Plant diseases associated with stubble tillage, however, have discouraged the continuance of these beneficial practices. In the United States, genetic resistance to diseases such as GLS is the long-term, most cost-effective solution to the problem (Lipps & Pratt, 1989), and is also likely to be the solution to GLS in South Africa. However, breeding for resistance is slow, and until hybrids resistant to the disease become commercially available, agronomic

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[1] Nutter, F.W., Department of Plant Pathology, Iowa State University, Ames 50011, U.S.A.

practices and the use of fungicides are essential for the management of the disease. These have been investigated in this thesis, and might provide short-term answers to the problem.

Because of the rapid rise in importance of GLS and the serious losses caused by it, this first investigation of GLS in South Africa was designed to establish solutions that could be easily and immediately implemented by farmers, as interim solutions to the problem. The approach of the following research was to:

(i) review available literature on GLS disease and related pathogens to assist in establishing possible solutions to the problem;
(ii) identify high-yielding commercial maize hybrids less susceptible to the disease;
(iii) investigate the interactive effects of tillage systems and fungicide treatments that minimise the effects of the disease;
(iv) evaluate fungicides for their correct and proper use for the control of GLS, and
(v) evaluate the financial benefit of fungicide treatment in South Africa for the control of GLS.

In order to establish solutions to the approach adopted, a series of trials were conducted at Cedara to:-

(i) Identification of high-yielding maize hybrids that are less-susceptible to GLS. (Maize hybrid response to grey leaf spot disease under two tillage systems in South Africa). The most widely grown maize hybrid before the advent of GLS in KwaZulu-Natal was GLS-susceptible RS 5206. With GLS, this hybrid was found to suffer yield losses up to 60%. The object of the trial was to identify high-yielding maize hybrids that are less-susceptible to GLS than RS 5206 and suitable for commercial agriculture under both stubble and conventional tillage practices. The trial design was stubble and conventional tillage as whole plot treatments, split for 49 hybrid sub-plot treatments in a 7 x 7 triple lattice design replicated three times. The trial was assessed for disease severity and grain yield. The data generated were used to calculate area under disease progress curve (AUDPC) which is a summary of the disease epidemic and, the disease data was transformed to fit the logistic model described by Vanderplank (1963). Fitted regression functions of the transformed data were used to estimate the number of days to 1% leaf-blighting. Disease severity and yield data were presented on a relative basis to remove effects of season. The Bestest analysis was developed and used for ranking hybrids into three subsets for disease susceptibility and grain yield, whilst regression analysis was used to further identify hybrid susceptibility. Days to 1% leaf-blighting was an added parameter to support the findings since disease is expected earlier in susceptible hybrids less-susceptible hybrids.

(ii) Having established high-yielding hybrids less-susceptible to GLS, the objective of the following trial was to establish if grain yields of hybrids could be increased using fungicides and to identify those hybrids with the most favourable responses to treatment. (Fungicide responses of maize hybrids to grey leaf spot disease). The same trial described in (i) above was used, but a further whole-plot treatment with fungicides was included. The same disease and grain yield assessments described in the above study were used. The data were presented on a relative basis to indicate responses of the hybrids to fungicide treatment. Regression analysis was used to study the effect of fungicides on disease severity and, the gain in yield due to fungicide treatment against disease severity of the hybrids tested. The data generated from the regression analysis enabled the prediction of yield responses of hybrids to fungicide treatment under varying levels of disease.

(iii) The beneficial effects of stubble on soil and moisture conservation under the inadequate rainfall regime in KwaZulu-Natal, and the financial benefits from reduced tillage, have led to a resurgence of interest in conservation tillage practices. However, increased disease is often associated with increased surface residues. This study was therefore initiated to investigate the interactive effects of tillage systems that leave varying amounts of stubble residues on the soil surface and fungicide treatments in reducing foliar diseases associated with debris. The study comprised four tillage treatments - no-till, chisel plough, chisel and disc, and mouldboard plough. The trial was a randomised complete blocks design and tillage treatments were split for fungicide-sprayed and unsprayed sub-treatments. Disease and grain-yield assessments were conducted and these data used to establish AUDPC, final disease severity, the logistic model, days to 1% disease, infection rates and grain yields. These data were used to study the advantages of stubble and ploughed tillage systems and the effect of fungicides on these in managing GLS.

(iv) As no maize hybrids had been identified with sufficient resistance to control GLS, fungicides were evaluated to establish products that would provide control of GLS. The objective of the study was to establish which fungicides and mixtures of fungicides with different modes of action were most effective in control of disease. A second experiment was initiated to establish optimum rates of application of promising fungicides. (Chemical control of maize grey leaf spot). Fungicides were selected on the basis of past performance against other species of *Cercospora* in other crops, and from recommendations by chemical manufacturers. Products selected included fungicides of the benzimidazole and triazole chemical groups. Trial designs were complete randomised blocks with three and four replications. Disease and grain yield assessments were conducted and used to calculate AUDPC values. Data were transformed to fit the logistic model and used to calculate the effective period of control, which is useful parameter to compare fungicide performance.

(v) The most effective time to commence fungicide treatment and the frequency of application are factors to consider in any spray programme, and are important to the success of treatment. The objective of the study was to establish the most effective time to initiate treatment and the frequency of treatments necessary to control disease until crop physiological maturity. Assessment of the trial and analysis of data was similar to that used in the evaluation of fungicides trial. AUDPC, effective period of control and grain yield data are important parameters in determining the correct time to initiate spray treatment and, the frequency of sprays necessary for effective control.

(vi) Having established that fungicides were effective in the control of GLS, it was appropriate to evaluate the economic justification for fungicide treatment. This was identified by calculating the economic benefits from data generated from single and multiple spray treatments in the "Frequency and timing of fungicide applications for the control of grey leaf spot in maize" trial. Economic analysis was based on average operating costs of a survey of 18 representative dryland maize farms, and an expected maize price of R400 ton^{-1}. The added profit due to fungicide treatment was calculated from the gain in yield due to fungicide treatment less the costs of fungicide and application, and the extra cost for harvesting the gain in yield. The added profit represented the economic benefit from fungicide use. Upper and lower limit added profit were calculated from 95% confidence limits of the gain in yield due to fungicide use. Decisions on the use of fungicides are based on the expectation that the financial return from investment in fungicide treatment will exceed cost of treatment.

The following thesis has been written in the form of several chapters, which cover various facets of the research conducted on GLS. Manuscripts of these facets have been submitted for publication and have been reviewed by editorial staff of the journals to which the papers have been submitted. At the time of writing, three manuscripts have been published. The remaining three manuscripts have been accepted for publication and are "in press". Published papers are:

Fungicide responses of maize hybrids to grey leaf spot. European Journal of Plant Pathology. 1996. 102:765-771. Authors: Ward, J.M.J., Hohls, T., Laing, M.D. and Rijkenberg, F.H.J.

Frequency and timing of fungicide applications for the control of grey leaf spot in maize. Plant Disease 1997. 81:41-48. Authors: Ward, J.M.J., Laing, M.D. and Rijkenberg, F.H.J.

The economic benefits of fungicide treatment of maize for the control of grey leaf spot (*Cercospora zeae-maydis*) in KwaZulu-Natal. South African Journal of Plant and Soil. 14:43-48. Authors: Ward, J.M.J., Darroch, M.A.G., Laing, M.D., Cairns, A.L.P. and Dicks, H.M.

Papers in press are:-

Chemical control of maize grey leaf spot. Crop Protection. Authors: Ward, J.M.J., Laing, M.D. and Nowell, D.C.

Management to reduce gray leaf spot of maize. Crop Science. Authors: Ward, J.M.J., Laing, M.D. and Cairns, A.L.P.

Paper accepted for publication:-

Maize hybrid response to grey leaf spot under two tillage systems in South Africa. South African Journal of Plant and Soil. Authors: Ward, J.M.J., Nowell, D.C., Laing, M.D. and Whitwell, M.I.

As the thesis follows the style and conventions required by the particular journal, the style and conventions used in the following chapters and list of contents differ from each other according to the individual journal requirements.

1.2 *LITERATURE REVIEW*

1.2.1 HISTORY AND DISTRIBUTION

Grey leaf spot (GLS), caused by the fungus *Cercospora zeae-maydis* Tehon and E.Y. Daniels, has become an increasingly important disease of maize (*Zea mays* L.) in South Africa. It was first identified as a disease of economic importance to the South African maize industry by Ward and Nowell (1997) in 1991. The disease is capable of reducing grain yields by as much as 60% in the more humid, high potential maize-growing areas, and reduces the yield and quality of sweetcorn and maize grown for silage (Ward & Nowell, 1997). Grey leaf spot has assumed epidemic proportions since the 1990/91 season and now results in significant yield losses each season. It has established itself, primarily, in the province of KwaZulu-Natal, but has been identified in the neighbouring provinces of Eastern Cape and Mpumalanga (Gevers & Lake, 1994; Ward & Nowell, 1997).

[2]Nutter (pers. comm., 1994) concluded, after visiting South Africa, that the potential for GLS to cause severe economic hardship in maize production was many times greater in South Africa than in the United States, because it occurs earlier and at higher levels. The pathogen was first identified in the United States by mycologists Tehon and E.Y. Daniels in 1924 (Tehon & E.Y. Daniels, 1925). From then until 1943, the disease remained relatively obscure (Arnt, 1943; Hyre, 1943; Lehman, 1944). Hyre (1943) was the first to observe the destructive potential of GLS, when he reported the incidence of the disease to be more than 90% in some counties in Kentucky, where disease severity levels ranged between 18% and 45%. He, however, did not quantify yield loss data. The disease was subsequently reported in North Carolina in 1949 (Kingsland, 1963) and in Virginia in 1949 and 1950 (Roane, 1950). Until the 1970s, GLS was considered to be of minor importance, and was largely limited to the humid mountainous areas of the eastern states of the United States, where it was associated with maize grown under monoculture and reduced tillage practices (Hilty, *et al.*, 1979; Latterell & Rossi, 1983; Roane, Harrison *et al.*, 1974). Leonard (1974) was among the first workers to recognise the importance of GLS as a disease of maize, and postulated its association with moderate temperatures, abundant rainfall and humidity. The disease has since moved westward from the eastern states and has increased in incidence and severity in the Mid-Atlantic and Mid-West regions and continues to move westward into new ecological niches. This increase in prevalence and severity is associated with the increase in the practice of minimum tillage (Anderson, 1995;

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[2] Nutter, F.W. Department of Plant Pathology, Iowa State University, Ames 50011, U.S.A.

Perkins, *et al.* 1995). GLS has now become one of the major yield-limiting diseases of maize in the United States (Ringer & Grybauskas, 1995; Thorson & Martinson, 1993). In areas where GLS is a problem, yield potential of diseased maize may be reduced by as much as 50% due to loss of photosynthetic area, increased lodging and premature death (Saghai Maroof, *et al.*, 1996).

Outside the United States, Chupp (1953) identified Peru, Colombia, Trinidad and Brazil as countries where GLS occurred. It has since been reported in Costa Rica, Mexico and Venezuela (Boothroyd, 1964; Latterell & Rossi, 1983). Grey leaf spot is also a recognised disease of importance of maize in South Africa and China (Coates & White, 1995; Ward & Nowell, 1997).

1.2.2 SYMPTOMS

The earliest report of GLS described symptoms to be spots on maize leaves 5 x 10 to 20 mm in size, some lesions being confluent and more extensive (Tehon & E.Y. Daniels, 1925). According to Chupp (1953) leaf spots were extended pale brown streaks or irregular grey to tan spots running parallel to the midrib, often with a brown or maroon border. More recent descriptions are that early symptoms of infection are pin-point sized lesions, surrounded by a yellow halo, which are easily observed when the leaf is held to the light (Stromberg, 1986). The lesions are slow to develop in comparison with other foliar pathogens and can take 14 to 28 days for full expansion (Beckman & Payne, 1982). On susceptible genotypes, mature lesions are distinctly rectangular in shape, 10 to 70 mm long and 2 to 4 mm wide, and are delineated by the veins on either side of the lesion. They are tan to pale brown in colour and assume a grey caste during sporulation (Ayres, *et al.*, 1985; Latterell & Rossi, 1983; Stromberg, 1986). The diagnostic features of GLS lesions are the clear edges along major leaf veins and the opacity of the mature lesion (Coates & White, 1995). The sharp edges are due to the inability of the fungus to penetrate the sclerenchyma tissue in the major veins of the leaf (Beckman & Payne, 1982). The opacity of the lesions is due to the formation of stromatic tissue and the dark hardened mycelium of the fungus in the sub-stomatal cavities (Latterell & Rossi, 1983).

Lesion type, number and size may vary between genotypes having different resistance reactions. Many susceptible inbreds display numerous necrotic lesions (Huff, Ayres & Hill, 1988; Lipps & Pratt, 1989) Resistant inbreds may show fleck-type lesions (Ayres *et al.*, 1985) and moderately resistant hybrids display chlorotic lesions (Roane *et al.*, 1974).

Stalk deterioration and increased lodging result when leaf blighting due to GLS is initiated early, and there is significant blighting of leaves during grainfill (Latterell & Rossi, 1983; Roane *et al.*, 1974; Stromberg & Donahue, 1986). This is due to a greater demand for carbohydrates from stalks and root tissue by developing kernels as a result of the decreased photosynthesis in diseased leaves, and the maize plant becomes more susceptible to stem- and root-rotting fungi leading to increased lodging (Dodd, 1980 a & b). Severe lodging can adversely affect mechanical harvesting and result in further losses in grain yield.

NB *

1.2.3 ROLE OF CERCOSPORIN

Cercosporin, a red pigment, is a non-host-specific toxin produced by several species of the genus *Cercospora* and has been implicated in disease development in plants (Blaney, *et al.*, 1988; Daub, 1982).

Cercosporin is structurally related to several photosensitizing compounds which produce either singlet oxygen or superoxide ions. These are extremely toxic to plant cells, causing oxidation of fatty acids, sugars, cellulosic materials and amino acids, and results in destruction of cell membranes. Cercosporin acts in the plant as a photosensitizing agent that sensitizes and kills plant cells when they are exposed to visible light (Daub, 1982; Daub & Hangarter, 1983; Lipps, 1987). Tissue from older maize is less sensitive to cercosporin but genotypic differences have not been observed (Gwinn, *et al.*, 1987).

1.2.4 THE PATHOGEN

Cercospora zeae-maydis was first described by Tehon & E.Y. Daniels (1925). They noted conidiophores, olivaceous to brown, lax but ascending, 3- to 8-septate, 70 to 90 x 4 µm and bearing a single apical geniscar. Conidia, hyaline, are distinctly obclavate, 4- to 10-septate, and 50 to 85 x 5 to 9 µm. Abundant fasciculae arise through nearly closed stomata. Chupp (1953) described *C. zeae-maydis* as having conidiophores that were 1 to 3 geniculate and conidia being curved, 3 to 10 septate and 30 to 95 x 5 to 9 µm. This differs somewhat from the original description. Kingsland (1963) also amended the original description of Tehon & E.Y. Daniels by finding the conidiophores to be 1 to 3 geniculate, sparingly septate, arising from stomata on both leaf surfaces, but in greater abundance abaxially, and the conidia to be hyaline straight or slightly curved. The average length of conidiophores and conidia are 61 and 77 µm, respectively.

The perfect state of *C. zeae-maydis* is an undescribed and unnamed species of *Mycosphaerella*, which was found in overwintering field specimens by Latterell & Rossi

(1983), but rarity of its occurrence and maturation indicated that it was not a source of inoculum in spring. Reports of *C. sorghi* being a causal agent of GLS have not been substantiated (Mulder & Holliday, 1974).

1.2.5 EPIDEMIOLOGY

Conidia produced in infected maize debris from the previous maize crop provide the primary inoculum to infect the newly-planted maize crop (Beckman & Payne, 1982; Latterell & Rossi, 1983; Payne & Waldron, 1983). Spore counts were highest in early afternoon but aerial concentrations of conidia remain low until sporulating lesions on maize plants are formed. These low conidial counts, however, are not the reason for initial slow development of disease. It is rather the absence of suitable environmental conditions that delay disease development in the early season (Payne & Waldron, 1983). High relative humidity (in excess of 90 to 95%), and temperatures of 22 to 30°C, are considered optimum for spore germination (Beckman & Payne, 1982; Rupe, *et al.*, 1982). In laboratory studies, Rupe *et al.* (1982) found that spores require at least 6 hours of continuous leaf wetness for germination and optimum conditions were 9 hours of leaf wetness, at temperatures between 18 and 25°C. The conidia were unable to survive wetting and drying during germination and must germinate in an uninterrupted period of wetness. Thorson (1989) also found germination *in vitro* to occur after 6 hours with free water. Beckman and Payne (1982), on the other hand, were able to germinate conidia on plants after 24 hours at 22 to 30°C, when the maize plants were exposed to high humidity by intermittent misting for a 12-hour period. Outside this temperature range germination decreased.

Several germ tubes, one from each cell, may emerge from each conidium (Latterell & Rossi, 1983). The germ tubes grow in a hydrotropic response toward stomata. This is common amongst many *Cercospora* species of a number of hosts, which require high relative humidity (90 to 95%) and temperatures of 20 to 30°C for infection (Rathaiah, 1977; Schuh, 1991; Thorson, 1989). This response in *C. beticola*, for example, was most evident at relative humidity 96 to 98%, and was least evident in saturated humidity. Under lower ambient relative humidity, the water vapour pressure in the stomatal cavity is higher than ambient, which explains the hydrotropism of germ tubes towards stomata (Rathaiah, 1977). *Cercospora zeae-maydis* appears to be no exception. Beckman and Payne (1982), found germ tube growth to have a positive tropism toward stomata under high relative humidity. A swelling of germ tube terminals was observed 2 to 3 days after inoculation (DAI), and abundant appressoria formed over stomata after 4 to 5 DAI. In the presence of free water on the leaf surface, however, stomatal tropism is reduced and appressorial formation is rare, and there is no penetration of the host tissue (Beckman & Payne, 1982; Thorson & Martinson, 1993). This contrasts with the continuous leaf wetness requirement for germination suggested by Rupe *et al*. (1982). A single conidium with germ tubes usually produces two to five appressoria over different stomata. Only one infection peg develops from a single appressorium 6 or 7 DAI. Internal colonisation is confined to the air spaces and inter-cellular spaces within the parenchyma tissue of the mesophyll. Hyphal growth is generally delimited by the sclerenchyma tissue surrounding the major veins (Beckman & Payne, 1982). Chlorotic dots are the first visual symptoms and are observed after 9 DAI. These elongate to form narrow lesion initials at 12 DAI. The characteristic mature lesions show 13 to 16 DAI (Beckman & Payne, 1982). In later studies, Beckman & Payne (1983) obtained sporulating lesions 11 to 25 DAI. Ringer & Grybauskas (1995) established the latent period to range from 14 to 19 days for susceptible hybrids to 16 to 22 days for moderately resistant hybrids.

Many foliar diseases of maize are initiated during the reproductive phase of host development. There are, however, differing opinions among research workers with regard to the physiological stage of host development and infection by GLS. Rupe *et al.* (1982) suggested plant maturity to be an important factor in the development of GLS, as initial symptoms often appear at anthesis. Gwinn, *et al.*, (1987) observed stomatal penetration by *C. zeae-maydis* hyphae was more frequent in physiologically older tissue compared to younger host tissue. However, earlier research conducted by Hilty *et al.* (1979) found that GLS was not necessarily associated with senescence as GLS symptoms were produced in the greenhouse on 2 to 3 week-old seedlings. Beckman & Payne (1982) also discounted maturity as a factor and found that neither plant-age nor leaf-age influenced plant susceptibility to GLS, as the latent period for infection was shorter on younger than older leaves.

The primary infection usually develops on the lower maize leaves and, when lesions mature, conidia are produced that serve as inoculum to infect upper leaves. As more lesions form, they may coalesce, and individual lesions become more difficult to distinguish, and blighting results (Stromberg & Donahue, 1986; Ward & Nowell, 1997). Sheath and stalk lesions occur on plants in severely infected fields and the fungus moves through the leaf sheaths into the stalks after the lesions have become well established on the sheaths. This damage to stalks may result in a high percentage of lodging (Shurtleff & Pedersen, 1991; Stromberg & Donahue, 1986), adding further to losses caused by stalk-rotting fungi that are favoured by the carbohydrate depletion in stalks (Dodd, 1980a).

McGee (1988) listed alternative hosts of GLS as being barnyardgrass (*Echinochloa* sp.) and *Sorghum* spp. These are alternate hosts of *C. sorghi* (Frederiksen, 1986), but their host status has not been substantiated for *C. zeae-maydis* (Mulder & Holliday, 1974). According to Stromberg & Donahue (1986) the host range is limited to maize. The pathogen is not considered to be seed-transmitted and there are no records of it being seed-borne (McGee, 1988; Richardson, 1990).

Cercospora zeae-maydis is a polycyclic, facultative pathogen (Chupp, 1953; Stromberg & Donahue, 1986) and the pathogen overwinters only in infected maize residues (Beckman & Payne, 1982; Latterell & Rossi, 1983). The increase in incidence and severity of GLS over the last two decades has been linked to continuous maize production (Anderson, 1995; Beckman & Payne, 1982; Latterell & Rossi, 1983; Thorson & Martinson, 1993), and conservation tillage practices that leave maize residues on the soil surface (Beckman & Payne, 1982; Hilty *et al.*, 1979; Kingsland, 1963; Payne, Duncan *et al.*, 1987; Rupe *et al.*, 1982; Stromberg & Donahue, 1986). Disease levels increase with the amount of residue (de Nazareno, *et al.*, 1993; Perkins *et al.*, 1995). Conservation tillage is described as any form of tillage that leaves at least 30% of the soil surface covered with crop residue. In the United States, government policies and economics favouring conservation tillage have led farmers to increase cropping areas under such tillage practices and probably have encouraged the increased incidence of GLS (Anderson, 1995). An indication of the increase in conservation tillage can be found in the 1994 National Crop Residue Management Survey (Anon., 1995b), in which the area under conservation tillage increased from 32,5% in 1990 to 40,5% in 1994. The disease has increased in the United States as the use of conservation tillage has increased (White, *et al.*, 1996. Fig. 1).

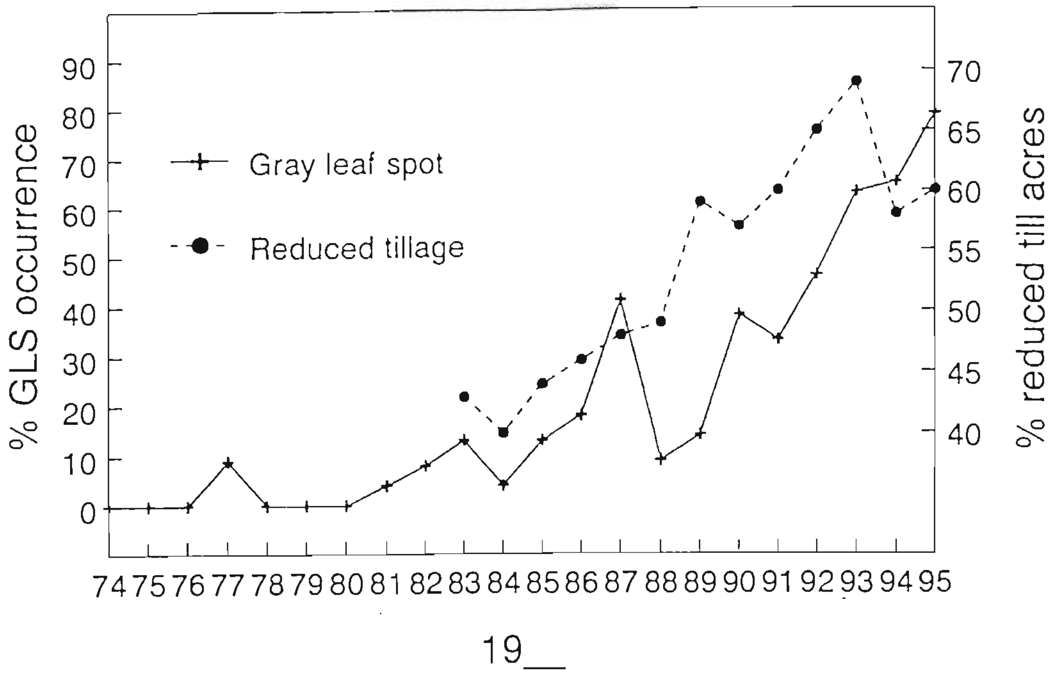

Figure 1. Occurrence (%) of gray leaf spot at DEKALB Genetics Corporation corn pathogen monitoring project plot locations and percent reduced tillage acres (> 15% residue cover) from the Conservation Tillage Information Center (cited by White, *et al.*, 1996)

Whilst stubble tillage is recognised as a valuable tool in conserving soil and soil moisture, its beneficial effects are frequently offset by the increased disease pressure from fungal pathogens, such as *C. zeae-maydis*, overwintering in the previous season's crop debris (de Nazareno *et al.*, 1993), which provides an earlier and more extensive source of inoculum (Payne *et al.*, 1987).

1.2.6 EFFECT OF ENVIRONMENT

The pathogen requires high humidity and cool cloudy conditions, with mists that extend the dew period (Anderson, 1995; Latterell & Rossi, 1983), but the exact conditions for disease development remain unclear. Conditions favouring GLS occur in mountain valleys and river bottoms (Payne *et al.*, 1987), areas in close proximity to water bodies (Ayres *et al.*, 1985) and in fields under irrigation (Hawke, *et al.*, 1985). Field studies by Beckman, *et al.*, (1981), indicate that high temperatures and low rainfall were not range limiting factors to GLS, and that microclimate with elevated relative humidity within the full canopy was an important factor in disease development. Latterell & Rossi (1983) suggested that the pattern of meteorological conditions associated with GLS damage had not been fully elucidated. High rainfall in the spring was not necessarily conducive to early onset of disease, as the disease could become 'dormant' if a dry summer followed. The authors could not explain why there was little GLS present in 1975, when rainfall was plentiful. In contrast, more recent work by Ringer & Grybauskas (1995) postulated that early rains created favourable environmental conditions contributing to relatively high numbers of primary lesions that may provide sufficient inoculum to cause subsequent high levels of disease severity. Low rainfall in these early infection cycles, on the other hand, may be a contributing factor affecting low numbers of primary lesions and lower levels of inoculum and disease. Rupe *et al.* (1982) also observed that high humidity was frequent in the two-week period prior to large increases in GLS severity. In South Africa, early rains have contributed to earlier and higher levels of disease (pers. obs.), whilst disease is observed later and only develops to relatively high levels after crop physiological maturity when seasonal rainfall is late or lower than normal. These observations support the findings of Ringer & Grybauskas (1995). The optimum temperatures for disease development were 22 to 30°C (Beckman & Payne, 1982; Latterell & Rossi, 1983). Studies in South Africa support these views (pers. obs.) as GLS was slow to develop on maize in KwaZulu-Natal province when mean temperatures were below 20°C.

In areas where inoculum had become generally abundant, Latterell & Rossi (1983) inferred that high humidity was not as critical to disease development where inoculum was less abundant. This is supported by Smith (1989). Perkins *et al.* (1995) also stressed the importance of abundant inoculum when they found that the disease became firmly established in new areas under minimum tillage, and was able to move from the stubble tilled lands with high levels of inoculum to become a problem in fields where conventional tillage was practised.

Unlike many other fungal pathogens, *C. zeae-maydis* is able to survive as stromata that form within the host tissue under adverse environmental conditions during early infection (Latterell & Rossi, 1983). Under adverse conditions, such as hot and dry periods, sporulation ceases and "bursts out" again to produce conidia with the return of favourable conditions. It is postulated that the stromata within the more humid substomatal cavity enable the pathogen to survive these external adverse conditions. The substomatal stromata need only brief exposure to moisture to produce conidiophores and conidia to reinfect the host (Latterell & Rossi, 1983).

1.2.7 MANAGEMENT OF GLS

An understanding of the epidemiology of *C. zeae-maydis* is important in the formulation of strategies for its control. It is a facultative saprophyte, and GLS is a polycyclic disease with a relatively long latent period. The pathogen is distinctly different from many other foliar pathogens of maize in that it requires a longer time to penetrate leaves, to produce lesions, and to produce secondary inoculum (White, *et al.*, 1996). The fungus may only complete a few cycles of secondary spread in a single growing season compared to the many cycles completed by most other corn leaf blight pathogens. Therefore, the severity of GLS in a particular geographical area is dependent on a large amount of overwintering and primary inoculum. If a tillage system leaves sufficient diseased crop residue on the soil surface, then enough primary inoculum may be available to result in severe levels of GLS (White *et al.*, 1996).

Reduction in the amount of initial inoculum is therefore the key to management of the disease. Reduction in the rates of disease development is an alternative means of managing GLS.

1.2.7.1 *Agronomic practices aimed at the reduction in the amount of initial inoculum*

The most damaging GLS epidemics in the United States occur in areas where reduced tillage practices allow the disease to become firmly established (Perkins *et al.*, 1995). However, the value of these practices in reducing soil erosion is unquestionable (Coates & White, 1995; Perkins *et al.*, 1995; Stromberg, 1986). Tillage operations aimed at complete burial of debris have been demonstrated as a means of managing GLS as the fungus dies within a few months of being buried in the soil. (Huff *et al.*, 1988; Payne & Waldron, 1983; White *et al.*, 1996). Discing, however, provides insufficient burial of residues (Stromberg, 1986), whilst ploughing can leave as much as 10% residue on the soil surface (de Nazareno *et al.*, 1993). This can provide sufficient inoculum to initiate an epidemic and subsequent tillage should be aimed at complete burial of the residual debris. Destruction of primary inoculum, however, is only feasible in areas where external sources of inoculum from adjacent infected fields are minimal (Smith, 1989).

There is no doubt that stubble tillage favours the incidence of GLS in the United States, but environmental benefits, economics and government policies will encourage the continuation of these practices. This means that the potential for the incidence of GLS will remain high (Anderson, 1995). In most instances this probably may not greatly reduce yields but, given the favourable conditions, severe losses could result (Perkins *et al.*, 1995).

Cercospora zeae-maydis does not survive much beyond 2 seasons in infected debris (de Nazareno, *et al.*, 1992), and, because it is host-specific, rotations to other crops such as soyabeans and cereals are an alternative to ploughing (Huff *et al.*, 1988; Latterell & Rossi, 1983; Stromberg, 1986). Crop rotations take longer to reduce inoculum levels than ploughing, usually requiring 2 years for the fungus to be reduced to low levels (White *et al.*, 1996). Rotating away from maize for two years with non-host crops in areas favourable for disease and where reduced tillage is practised, or one year under clean ploughing, are recommended (Spink & Lipps, 1987). In view of other pests and diseases such as eyespot, and ear- and root-rots associated with maize stubble, rotations are an attractive alternative especially in lower risk situations (Latterell & Rossi, 1983). However, Payne *et al.* (1987) pointed out that rotations are not always economically attractive and, historically, this has been the case in South Africa.

If a region has a large percentage of land in conservation tillage, maize in conventionally tilled fields or maize in rotation with soyabeans may be damaged by GLS as a result of inoculum disseminated from fields where conservation tillage is used. Maize debris in fields planted to soyabeans also is an important, and sometimes unrecognized, source of inoculum, that may infect the maize crops (White *et al.*, 1996).

Harvesting the maize for silage reduces the inoculum carry over to the next crop, since much of the infected foliage is removed before the disease becomes too severe (Stromberg, 1986). In areas where no-till is practised, lands previously cut for silage could be planted under stubble tillage, whilst land harvested for grain with ensuing debris could be ploughed (Payne *et al.*, 1987).

1.2.7.2 *Practices aimed at the reduction of disease development*

It was only in the 1970s, when GLS was recognised as being a threat to maize production, that efforts were made to find resistance to the pathogen. The first efforts to screen maize hybrids for resistance did not succeed, as all hybrids were found to be susceptible to the disease (Roane *et al.*, 1974). Hilty *et al.* (1979) found little resistance in hybrids, and only one inbred had a high degree of resistance to GLS in Tennessee. Since then, there have been reports of varying degrees of resistance to GLS (Ayres *et al.*, 1985; Coates & White, 1995; Gevers & Lake, 1994; Graham, *et al.*, 1994; Ramkey & Hallauer, 1993; Lipps & Pratt, 1989; Roane & Donahue, 1986; Stromberg, 1986). Currently-available hybrids in the United States display different levels of susceptibility to the disease, but few can be considered resistant (Perkins *et al.*, 1995). Host-resistance is considered one of the best options for managing GLS, as there are good sources of resistance and utilising resistance is simple for the grower (Coates & White 1995; Graham *et al.*, 1993; White *et al.*, 1996).

It is important to determine the level of resistance required in breeding programmes. This is to ensure new hybrids have advances in yield and other sound agronomic traits as well as reduced risk of yield loss from GLS. The addition of GLS resistance, as another criterion in the breeding programme, will reduce the number of hybrids that meets the minimum performance level for yield and other traits in the programme. Too high a requirement for GLS resistance will reduce the number of new hybrids released with superior yield, whilst too low a requirement for GLS resistance, with high yield potential, will put the producers at risk, where conditions are favourable for disease (Anderson, 1995).

Usually resistance to GLS is polygenic and additive in nature (Anderson, 1995; Ayres *et al.*, 1985; Bubeck, *et al.*, 1993), but there are reports of resistance being dominant (Gevers, *et al.*, 1994; Ellwinger, *et al.*, 1990). Rate-reducing polygenic resistance acts by adding small increments of resistance, which lead to an improvement in the level and stability of resistance. On the other hand, major gene resistance depending on a single gene can be overcome by a single gene mutation in the pathogen and, for this reason, breeding for resistance on a polygenic basis is theoretically desirable (Ayres *et al.,* 1985; Latterell & Rossi, 1983).

Recent developments with molecular genetic markers such as DNA restriction fragment length polymorphism (RFLP) has made it possible to construct detailed linkage maps, and to dissect the genetic control into quantitative traits. Genes controlling a quantitative trait must be identified; to determine the effect of these genes or quantitative trait loci (QTL's), to study the molecular mechanisms of individual genes and, more directly, to facilitate the transfer of desirable traits such as GLS resistant genes, in marker-assisted breeding programmes (Saghai Maroof, *et al.*, 1996). The authors suggest that the quantitative resistance to GLS might be controlled by a small number of genes or QTL's which appear on chromosomes 1, 2, 4 and 8, and that genetic control of GLS resistance is not very complex. Use of marker-assisted breeding programmes may hasten the development of acceptable GLS-resistant hybrids.

Maturity group, however, is an important parameter to consider in regard to losses from GLS. Later-maturing hybrids, although adapted to longer growing seasons and producing potentially higher grain yields, are at greater risk from GLS as they are subjected to blighting longer during a greater portion of their grain-filling period in the United States (Stromberg & Donahue, 1986). Hybrids with a shorter growing season should therefore be selected in areas where GLS is a problem.

Fungicides have been used to delay the development and severity of GLS in the United States (Stromberg, 1986). However, only surface protectant, non-systemic fungicides have until recently been registered for use on commercial maize in the United States and must be applied as a series of preventive treatments at 7- to 10-day intervals for effective disease control (Thorson, 1989). The cost of fungicide and its application is therefore high and, for this reason, chemical control of GLS on commercial maize crops is not an acceptable method of control (Lipps & Pratt, 1989). They add, however, that in years highly favourable for disease spread, chemical control may be a way of preventing excessive yield losses. Hybrid maize seed is a commodity of high economic value and seed producers often apply fungicides to reduce yield losses due to various foliar fungi (Rivera-Conales, 1993). Rivera-Conales (1993) adds that propiconazole, a systemic fungicide has proven efficacy in the control of foliar diseases of maize and was subsequently registered for use in maize in 1995. It could be more economically attractive than protectant fungicides, as fewer spray treatments are necessary. Propiconazole, however, may only be applied until the tassel stage of development.

In South Africa, systemic fungicides are registered for the control of GLS on maize and, having a "curative" action, can be applied after the onset of the disease, to provide cost-effective control. The object of the fungicide programme is to delay the rate of disease development until the grain is physiologically mature (Ward & Nowell, 1997). The effectiveness of the programme depends on the correct timing and application of sprays and, when correctly carried out, the programme is cost-effective (Ward, *et al.*, 1997). Chemical control of GLS in South Africa has become widely accepted by farmers in areas in which GLS is a problem, and until resistant hybrids are developed for commercial use, fungicides are likely to be widely used (Ward & Nowell, 1997).

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CHAPTER 2

Maize hybrid response to grey leaf spot under two tillage systems in KwaZulu-Natal, South Africa(1)

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Abstract

Grey leaf spot (GLS) of maize has seriously decreased grain yields in the province of KwaZulu-Natal, South Africa, and has been identified in neighbouring provinces and countries. The susceptibility of commercial hybrids to the disease was assessed under conventional and stubble tillage systems. The hybrids that were most susceptible to grey leaf spot had lowest yields under both tillage practices. Linear regression of relative yield against relative disease severity identified PAN 6479, PAN 6480 and CRN 3584 as high yielding maize hybrids that were less susceptible or tolerant to the disease. The development of Gupta's Bestest, ranking hybrids in subsets for disease susceptibility and yield performance, supported results obtained from linear regression analysis. There were no differences in grain yields between conventional (6177 kg ha^{-1}) and stubble (6321 kg ha^{-1}) tillage systems, indicating that the beneficial practice of stubble tillage can be used in areas where grey leaf spot is present, in a trial conducted on one site in KwaZulu-Natal.

Introduction

Grey leaf spot (GLS), a foliar disease of maize (*Zea mays L.*) caused by the fungus *Cercospora zeae-maydis* (Tehon and E.Y. Daniels, 1925), was first observed at Greytown, South Africa, during the 1988/89 season, and at Cedara in 1992. It has since spread throughout the province of KwaZulu-Natal and has been identified in neighbouring provinces and countries. GLS is capable of reducing grain yields by as much as 50 to 60% in the more humid, high potential mist-belt bioclimate of KwaZulu-Natal (Ward and Nowell, 1997). It also reduces the yield and quality of maize grown for silage. Nutter (pers. comm., 1994), following a visit to South Africa, concluded that GLS has a higher potential for reducing maize yields in South Africa than in the United States.

Stubble tillage offers maize farmers many advantages to the environment by reducing soil erosion and water loss and enables a lower cost of production. However, increases in the severity and distribution of GLS in the United States have been associated with no-tillage maize (Hilty *et al.*, 1979; Latterell and Rossi, 1983; Leonard, 1974; Roane *et al.*, 1974; Stromberg, 1986). Recently, the disease has been observed to move from reduced tillage situations to fields where traditional conventional tillage practices were used (Perkins *et al.*, 1995). Yield losses are most severe under monoculture maize and crop rotations have been found to offer an attractive means of control (Huff, *et al.*, 1988; Latterell & Rossi, 1983, Stromberg, 1986). In South Africa, maize has traditionally been grown under a system of monoculture and few farmers practise any form of rotational cropping (Channon and Farina, 1991). Rotations, however, are unlikely to be used as a means of control since farmers are reluctant to change cropping practices. Genetic resistance, therefore, is a highly efficient and cost-effective method of GLS control (Lipps and Pratt, 1989) and is the long-term solution to the problem. Sources of genetic resistance have been identified in the United States (Huff *et al.*, 1988), but the germplasm is not well adapted in South Africa. Detailed genetic investigations have identified resistant genotypes in South Africa (Gevers *et al.*, 1994, Hohls *et al.*, 1995). However, no commercially available hybrids resistant to GLS have so far been released in South Africa, and chemical control methods are being used as an interim solution (Ward *et al.*, 1997). A resistant hybrid has the ability to exclude or overcome, completely, or in some degree, the effect of the pathogen, whilst a susceptible hybrid lacks this ability to resist disease. On the other hand a tolerant hybrid is one which has the ability to sustain the effects of disease without suffering serious crop loss (Agrios, 1988).

The purpose of this study was to evaluate and identify high yielding maize hybrids that were less susceptible or tolerant to GLS. Hybrids were evaluated under stubble and conventionally ploughed systems of tillage, to identify those hybrids best suited to each form of tillage.

Materials and methods

Trial data

The trial was conducted at the Cedara Agricultural Development Institute at Cedara, (29°31'S, 30°17'E and alt. 1070 m), and at Pannar (Pty.) Ltd., near Greytown (29°02'S, 30°31'E and alt. 1100 m), in South Africa. Maize had previously been grown on the sites before the National Maize Hybrid Cultivar Trial commenced in 1982.

Hybrids were evaluated for susceptibility to GLS during the 1991/92, 1992/93 and 1993/94 growing seasons. The trial at Cedara included conventional and stubble tillage systems laid out as whole plot treatments which were split for 49 hybrid sub-plot treatments in a 7 x 7 triple lattice design. The treatments were replicated three times. At Greytown the trial was conventionally tilled only. The sites at both locations were gently sloping and soils were well-drained sandy-clay loams of the Hutton form and Doveton series (MacVicar, 1991). The conventional-ploughed treatment was disced in the winter, mouldboard ploughed in September and finally disced immediately before planting to incorporate the previous season's maize residue. The stubble treatment was chisel-ploughed to a depth 120-mm in the winter and again prior to planting. Chisel-plough tines were spaced 310-mm apart and fitted with sweeps. Stubble residue on the soil surface prior to planting was calculated using a siting frame described by Lang and Mallett (1982). Residue cover on stubble treatment was 31%. Planting lines were drawn immediately prior to planting when fertilizer sufficient for an eight-ton grain crop was band applied. A topdressing of 100 kg N ha^{-1} was broadcast when maize was knee-high. Normal weed and pest control practices were followed for the two growing regions. Hybrids were planted in plots of two, 6.6 m rows spaced 0.75 m apart at Cedara. In-row plant spacings were 0.30 m. The trial plots were surrounded by two border rows to ensure that there were no microclimate effects between treatments. This is an accepted design used in South Africa and Virginia, US., for disease evaluation in hybrid trials. The trials were jab-planted by hand in early November each season and two seeds per planting station were planted. Thirty days after planting, the seedlings were thinned to 44 400 plants ha^{-1}. Two, 6.0 m rows, were hand-harvested to estimate yield. At Greytown, plots were two rows, 6.0 m long and 0.91 m wide, and hand-planted in early October, and thinned to 50 000 plants ha^{-1}. Two, 5.4 m rows were hand-harvested to estimate yield.

Weather

Weather conditions differed markedly over the seasons in which the experiments were conducted. Rainfall and temperature in 1991/92 were favourable for vegetative growth of maize until anthesis, after which, at the end of February and during grain-fill, rainfall declined. However, heavy dews were frequent during grain-fill, which favoured disease development. The 1992/93 season was dry, with only 50% of the mean expected rainfall being recorded during the growing season. In contrast, the rainfall during the 1993/94 season was above average and well distributed throughout the growing season. Mists were abundant, especially during grainfill in January and February.

Cultivars

Commercially available hybrids tested in the South African National Cultivar Phase II series of trials were studied during the seasons of 1991/92, 1992/93 and 1993/94. The results of the evaluations made for conventional tillage treatments in 1992/93 were discarded because of low disease levels induced by the prevailing drought and the resultant heterosedasticity of variance.

Disease and grain yield assessments

Whole-plant standard area diagrams described by Ward *et al.* (1997) were used as a guide in estimating disease severity (%). Assessments were made regularly on plants in the centre of each plot. In 1991/92, plots were assessed three times for GLS: at 60, 102 and 127 days after planting (DAP). In the following seasons, plots were assessed at first signs of disease and thereafter at approximately 14-day intervals. In 1992/93, five assessments were made and in 1993/94 there were four assessments. These data were used for calculating the area under the disease progress curve (AUDPC), which provides a summary of the disease epidemic. The AUDPC was calculated using a trapezoidal integration program (Berger, 1981). The AUDPC parameter was standardised by dividing the AUDPC value by the durations of the epidemic to enable comparisons between epidemics of different durations to be made. The standardised AUDPC was compared to critical (single) point models of disease severities rated between 120 and 130 DAP. Correlations between these two methods were highly significant and varied from 0.994 in 1991/92 to 0.889 in 1993/94. The single point model (% disease severity) was used as the disease index in the linear regression analysis. Disease severity data for nine hybrids, representative of different GLS susceptibility groups under conventional and stubble tillage at Cedara and Greytown, were transformed to fit the logistic model described by Vanderplank (1963). The fitted regression functions of the transformed values were used to estimate the number of days between planting and 1% leaf blighting. Relative disease severities were calculated by dividing disease severities by the trial mean, expressed as a percentage. Grain yields were expressed in kg ha^{-1} at 12.5% moisture. Relative yields were calculated by dividing grain yields by the trial mean, expressed as a percentage. Disease severities and yields have been presented on a relative basis to remove effects of season and location. (Stromberg and Donahue, 1986).

Statistical analysis

Eighty-five hybrids were evaluated at Cedara and Greytown. However, only data from 24 of the hybrids (Table 1), common to the three years of study, were used in the analysis of variance.

Table 1. Actual and relative disease and yield of 24 maize hybrids under stubble and conventional tillage systems

Hybrid	Stubble Tillage$^{(a)}$				Conventional Tillage$^{(b)}$			
	Disease		Yield		Disease		Yield	
	Actual $^{(c)}$ %	Relative$^{(d)}$ %	Actual kg ha^{-1}	Relative$^{(e)}$ %	Actual$^{(c)}$ %	Relative$^{(d)}$ %	Actual kg ha^{-1}	Relative$^{(e)}$ %
CRN 3584	**24**$^{(f)}$	**44**	**6970**$^{(g)}$	**116**	**28**$^{(h)}$	**113**	**7754**$^{(i)}$	**111**
PAN 6479	**25**	**47**	**7037**	**116**	**17**	**33**	**8108**	**115**
PAN 6480	**35**	**66**	**7242**	**121**	**24**	**49**	**8947**	**128**
PAN 6578	37	66	6579	108	30	66	7396	104
SNK 2665	37	66	6162	101	35	73	7139	100
PAN 6363	38	77	6707	111	40	81	8387	118
NS 9100	38	77	6094	100	30	67	7372	104
TX 24	39	78	6761	112	37	74	7590	107
PAN 6549	**43**	**82**	**6182**	**101**	**32**	**63**	**7428**	**104**
SNK 2021	44	86	5381	89	47	108	7023	99
CRN 4502	46	91	5809	95	48	118	6977	98
PAN 6364	47	93	6121	101	40	92	7381	104
RO 413	51	112	5642	93	39	79	6706	95
SNK 2888	**52**	**113**	**6550**	**108**	50	121	**7587**	**108**
CRN 3414	53	113	5934	95	45	110	6883	96
RO 430	55	127	5203	85	50	121	6271	87
SNK 2950	**57**	**131**	**6145**	**101**	**54**	**126**	**7307**	**102**
PAN 6528	**57**	**131**	**5236**	**85**	**52**	**120**	**6505**	**89**
CRN 4523	57	131	5488	90	56	137	6633	92
A 1849	58	142	6088	100	46	96	6220	103
CRN 4605	61	150	5473	89	57	139	6148	85
RS 5206	**63**	**152**	**5704**	**93**	**53**	**91**	**7148**	**99**
RS 5232	63	153	5338	85	65	169	5868	81
PAN 6552	**66**	**166**	**5492**	**91**	61	164	7220	100

(a) Mean performance of 24 hybrids evaluated at Cedara over 3 seasons 1991/92, 1992/93 and 1993/94.

(b) Mean performance of 24 hybrids evaluated at Cedara and Greytown over two seasons 1991/92 and 1993/94.

(c) Actual disease is percent leaf-blighting assessed approximately 21 days before physiological maturity.

(d) Relative disease is calculated by dividing disease percent by the trial mean and multiplying by 100.

(e) Relative yield is calculated by dividing the yield by the trial mean and multiplying by 100.

(f) Least susceptible hybrid subset ranked by Bestest analysis have <35% disease and most susceptible hybrids have >56% disease.

(g) Highest yielding hybrids ranked by Bestest analysis have >6580 kg ha^{-1} and lowest yielding hybrids have <6000 kg ha^{-1}.

(h) Least susceptible hybrids ranked by Bestest analysis have <35% disease and most susceptible hybrids have >47% disease.

(i) Highest yielding hybrids/ranked by Bestest analysis have >7600 kg ha^{-1} and lowest yielding hybrids have <7030 kg ha^{-1}.

Analysis of variance

Bartlett's χ^2 test was used to establish homogeneity of variances (Gomez and Gomez, 1984). The combined analysis of disease data from Cedara and Greytown was weighted by the inverse of the error mean square as disease heterogeneity of variance was present. Hybrid standard error of a mean was calculated using hybrid season interaction mean square for the analysis of different seasons (Gomez and Gomez, 1984). Analysis of variance was conducted using Genstat 5.2 (Anon., 1987).

The Bestest analysis (Gupta, 1965), developed by Calitz (1991), and van Aarde (1993, 1994), was used to rank hybrids into highest yielding subsets. By using the inverse of the data, hybrids were also ranked into lowest yielding subsets. Combining the analyses, hybrids were grouped in highest-, intermediate- and lowest yielding subsets. The hybrids were similarly grouped for high-, intermediate- and least severities for disease. Both groupings were based on an $\propto$-level of significance ($\propto > 0.05$).

Regression analysis

A linear regression model described by Stromberg and Donahue (1986), was used to determine the effect of GLS on relative disease on grain yield and relative yield.

The effect of GLS on grain yield and relative yield was estimated by the linear regression model:

$$Y = B_o + B_i XI + E_i$$

where Y is the response variable (yield), B_o is the intercept (yield when disease is zero), B_i is the slope of the regression line (regression coefficient or change in yield per unit change in disease), XI is the regressor variable (disease intensity at a particular stage) and E_i is the unexplained variation (error or residual). Regression lines were fitted for stubble-, and conventional-tilled treatments. Confidence limits (95%) were calculated for each regression line. The regression analysis was conducted on Genstat 5.2 (1987) and Statgraphics 4.0 (Anon., 1989).

Correlation analysis

Phenotypic and genotypic correlations were calculated to gain further insight into the relationship between GLS and yield. The appropriate variance and covariance components were determined through residual maximum likelihood analysis of the data.

Results

Disease severity

Effects of tillage at Cedara

Disease levels were relatively low in the 1992/93 season due to the prevailing drought, being 3.80% and 32.92% (±0.67) for conventional and stubble tillage respectively. Tests for homogeneity of variance, using Bartlett's χ^2 test over the three seasons, indicated variance to be heterogeneous ($\chi^2 = 47.9$, $P<0.001$). The same test over the 1991/92 and 1993/94 seasons showed the variances to be homogeneous ($\chi^2 = 0.462$, N.S.), and the results of 1992/93 are therefore excluded from the analysis. (Only where stubble treatments were considered on their own were the 1992/93 data included).

There was no interaction between tillage and season, indicating that tillage treatments affected disease levels consistently over the 1991/92 and 1993/94 seasons. There were no significant differences in disease levels between conventional and stubble treatments (Table 2).

Table 2. Effect of conventional and stubble tillage treatments on grey leaf spot disease severity and yield of 24 maize hybrids at Cedara and Greytown over 1991/92 and 1993/94 seasons

Tillage/Location	**Season**		
a) Disease severity (%) [a]			
Tillage	1991/92	1993/94	Mean
Conventional	32.08	67.57	49.83
Stubble	26.42	83.68	55.05
F-probability (P,0.05)[b]	NS	NS	NS
Standard error	1.55	7.44	3.80
CV%	37.1	15.4	21.5
Location			
Cedara	32.08	67.57	49.83
Greytown	14.69	59.01	36.85
F-probability ($P<0.05$)	*	NS	*
Standard error	3.50	8.90	3.80
CV%	33.4	14.9	11.9
b) Grain yield (kg ha^{-1})			
Tillage	1991/92	1993/94	Mean
Conventional	7557	4798	6177
Stubble	8161	4480	6321
F-probability ($P<0.05$)	*	NS	NS
Standard error	78.35	157.15	326.07
CV%	6.8	11.6	8.6
Location			
Cedara	7557	4798	6177
Greytown	9389	6922	8156
F-probability ($P<0.01$)	**	**	**
Standard error	95.80	85.10	64.10
CV%	8.0	8.9	8.4

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[a] Disease severity is percent leaf-blighting of whole plants, assessed approximately 25 days before crop physiological maturity

[b] F-probability ($P<0.05$) NS differences are not significant
* differences are significant
($P<0.01$) * differences are highly significant

Effects of location

A weighted analysis was used to compare average disease levels over the two seasons because of heterogeneous variances (χ^2 = 65.45, $P<0.001$). There was consistently more disease at Cedara (49.83%) than Greytown (36.85%), under conventional tillage (Table 2).

Grain yield

Effects of tillage

Grain yields for 1991/92 and 1993/94 are presented (Table 2). The yields of conventional tilled plots in 1992/93 were 4648 kg ha^{-1} for stubble plots. Stubble tillage practices in the 1991/92 and 1992/93 seasons had higher grain yields than conventional tillage ($P<0.05$). In 1993/94, with above average and well distributed rainfall and higher disease levels, there were no significant differences in yields of stubble and conventional tillage systems (Table 2). Tests for heterogeneity of variance over the three seasons showed variances to be homogeneous (χ = 1.378 N.S.). Over all seasons there were no differences between tillage practices.

Effect of location

Variances over the two locations for 1991/92 and 1993/94 seasons were homogeneous (χ^2 = 4.94, N.S.). There was no interaction between locations and seasons, indicating that yields were affected similarly by GLS at both locations over seasons. The overall grain yield at Greytown was 8156 kg ha^{-1} which was higher than at Cedara 6177 kg ha^{-1} (Table 2).

Effect of grey leaf spot on grain yield

Grain yield of 79 hybrids was regressed against disease severity for the 1991/92 and 1993/94 seasons and locations under stubble and conventional tillage treatments. The model accounted for 66.9% of the overall variation (significant $P<0.001$), an intercept of 9466 ± 123 kg ha^{-1} and a slope of -55.85 ± 2.27. There was no interaction between GLS and tillage treatments, indicating that the effect of GLS on yield is similar under both tillage treatments. There was no interaction between GLS and locations indicating that GLS affected yields similarly at both locations. There was also no tillage season interaction and, the effect of tillage practices on GLS, affected yields consistently over seasons in the regression model. There was, however, a significant interaction of location and season, and disease severity and season. The final model including differences in location, tillage season and the significant interaction of disease and season, accounted for 80.3% of variation.

Hybrid response to GLS

Disease severity and yield of 24 maize hybrids are presented on a relative basis (Table 1). This has been done to remove the effects of season and location to allow comparisons of hybrids across seasons and locations. Nine high yielding hybrids, representative of the different GLS susceptibility groups, were selected for ease of presentation. The yields of these hybrids were similarly high and over 8.0 ton ha^{-1} in the absence of GLS in fungicide sprayed studies and all exceeded the trial mean over the 1992/93 and 1993/94 seasons except for PAN 6364, which was 98% of the trial mean. (Ward *et al.*, 1996). PAN 6479, PAN 6480 and CRN 3584 were least susceptible to GLS. PAN 6528, PAN 6552 and RS 5206 were most susceptible, whilst PAN 6364, SNK 2888 and SNK 2950 were of intermediate susceptibility. This was confirmed by Bestest ranking of hybrids for disease severity and grain yields (Table 1). PAN 6479, PAN 6480 and SNK 3584 were the least susceptible subset and had the highest grain yields, whilst PAN 6528, PAN 6552 and RS 5206 were in the most susceptible subset to GLS.

Overall, hybrids with low GLS had high grain yields under both conventional and stubble treatments than hybrids with high disease. Except for Greytown, where disease levels were lower than Cedara, the percentage variance accounted for was highly significant in the regression of relative disease against relative yield for hybrids and seasons under both tillage treatments (Table 3). Under conventional tillage the less susceptible hybrids had lower than the trial mean relative disease and the hybrids yielded as predicted by the model, except PAN 6480, which yielded higher than predicted (Figure 1 a and c). Of the susceptible hybrids with more GLS than the trial mean hybrids, RS 5206 yielded as predicted, whilst PAN 6552 was higher than, and PAN 6528 had lower than, the predicted yield. The hybrids with intermediate susceptibility to GLS, SNK 2888 and SNK 2950 had higher than predicted yields, whilst PAN 6364 had yields close to that predicted by the model (Figure 1 a and c).

Table 3. Response of relative grain yield to relative grey leaf spot [(a)] of maize, representing 24 hybrids under conventional and stubble tillage at Cedara and Greytown during two to three seasons

Location	Tillage	Regression parameters		% variance accounted for (R^2)
		Intercept	Slope	
Cedara	Conventional	131.26 ± 6.59	-3.0870 ± 0.0622	50.7**[(b)]
Greytown	Conventional	110.45 ± 5.08	-0.0961 ± -0.0470	11.9 NS
Cedara	Stubble	122.97 ± 4.28	-0.2268 ± 0.0388	59.0**

[(a)] Values expressed as a percentage of the trial mean

[(b)] ** highly significant ($P<0.01$)

NS not significant

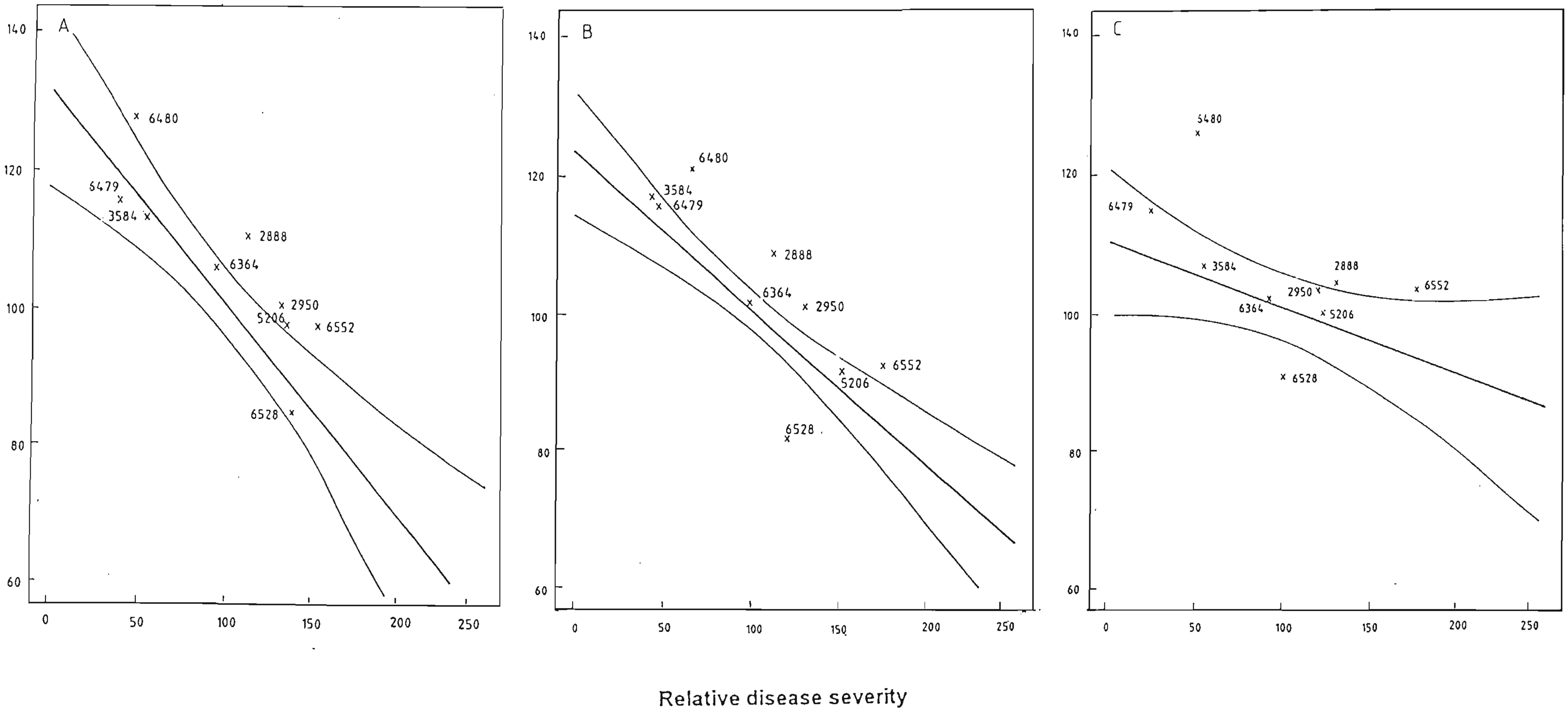

Figure 1. Regression analyses of relative grain yield against grey leaf spot relative disease severity at Cedara and Greytown under two tillage systems: (A) Cedara conventional tillage; (B) Cedara stubble tillage and (C) Greytown conventional tillage. Confidence limits (95%) are shown. The means of only nine of the hybrids regressed are shown. P6479, P6480 and C3584 are amongst the least susceptible, P6528 and P5206 are amongst the most susceptible, S2888 and P6552 is tolerant and P6364 and S2950 are intermediate in their reactions to grey leaf spot.

The pattern of hybrid response under stubble tillage was similar to that under conventional tillage, with PAN 6480, SNK 2888, SNK 2950 and PAN 6552 having higher relative yields than predicted, whilst PAN 6528 had lower than predicted relative yields. (Figure 1 b).

Data of the nine selected hybrids was grouped in less susceptible, intermediate and highly susceptible disease categories. Yield regressed against log-transformed disease, for seasons and locations under stubble and conventional tillage. When the effect of hybrid group was included in the model, there were significant interactions between disease severity and hybrid group (R^2 = 53.3, $P<0.001$) (Figure 2). Genstat pair wise test confirmed the presence of these interactions, namely that there were significant differences between the susceptible and less susceptible and intermediate susceptible groups ($P<0.05$).

Effect of hybrid susceptibility to GLS on the onset of disease (1%) disease (standardised AUDPC) and grain yields

With less susceptible hybrids, disease development took longer to reach 1% leaf-blighting than the group of hybrids susceptible to GLS (mean days to 1% disease for less-susceptible hybrids in 1991/92 was 77 days and for susceptible hybrids was 58 days. In 1992/93 this was 107 days for less-susceptible and 99 days for susceptible hybrids and in 1993/94 this was 79 days for less-susceptible and 76 days for susceptible hybrids) (Table 4).

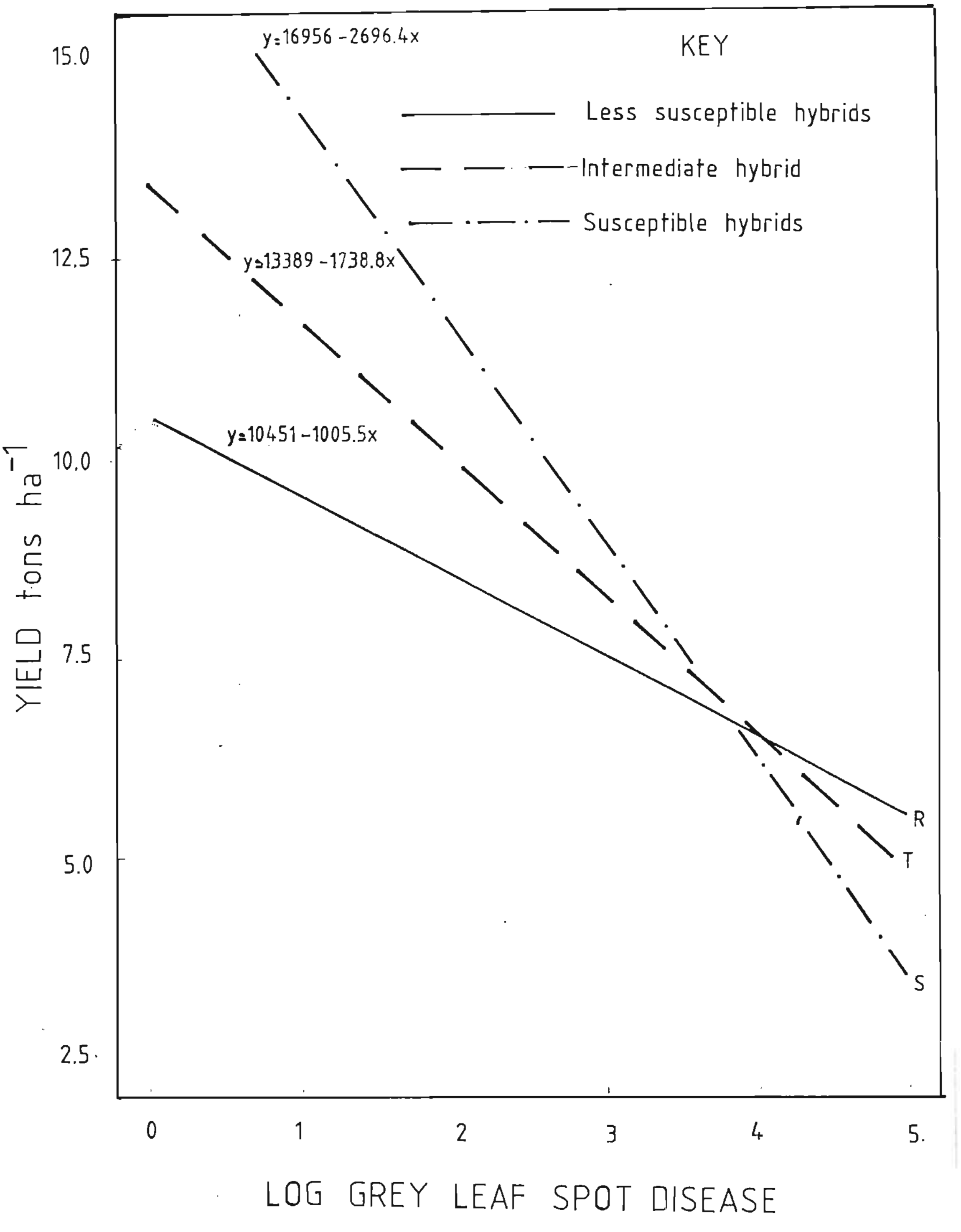

Figure 2. Regression analysis of grain yields against log-transformed grey leaf spot disease severity of less-susceptible, intermediate and susceptible groups of hybrids. The hybrids were evaluated over two and three seasons across two locations under stubble and conventional tillage systems. Less-susceptible hybrids were PAN 6479, PAN 6480 and CRN 3584. Intermediate hybrids were PAN 6364, SNK 2888 and SNK 2950. Most susceptible hybrids were PAN 6528, PAN 6552 and RS 5206.

Less susceptible hybrids had significantly lower disease (AUDPC) in all seasons than susceptible hybrids, and except for the drought season of 1992/93, these lower disease levels were reflected in higher grain yields than susceptible hybrids. (Table 4).

There were no differences in days to 1% disease between hybrids under stubble and conventional tillage in the wet seasons. But in the dry season of 1992/93, disease reached 1% disease earlier in the stubble treatments (94 DAP) than in conventional tillage (119 DAP) ($P<0.001$). There were no differences in yields obtained under stubble or conventional tillage in the three seasons of study.

The significant correlations of observed GLS disease ratings of hybrids (phenotypes) and grain yield, indicated a negative correlation between GLS and grain yield. This confirmed that the most susceptible hybrids (genotypes) had the lowest grain yields (Table 5).

Table 4. Days after planting (DAP)[a] to 1% disease, standardised AUDPC and grain yields for nine maize hybrids under two tillage systems at Cedara over three seasons

		DAP to 1% disease			AUDPC[b]			Yield (Kg ha^{-1})		
		Tillage[c]								
Season	**Hybrid**	Conv	Stub	Mean	Conv	Stub	Mean	Conv	Stub	Mean
91/92	PAN 6552	58	64	61	33.0	30.0	31.5	7431	6934	7182
	RS 5206	60	52	56	26.5	25.0	25.8	8073	8433	8254
	PAN 6528	58	57	57	28.6	19.8	24.2	7253	7183	7218
	MEAN	59	58	58	**29.4**	**24.9**	**27.2**	**7586**	**7517**	**7555**
	SNK 2950	65	53	59	24,8	21.1	23.0	7662	8463	8062
	PAN 6364	65	70	68	11.8	10.0	10.9	8183	8123	8153
	PAN 2888	70	59	65	18.3	13.8	16.1	7515	8517	8016
	MEAN	**67**	**61**	**64**	**18.3**	**15.0**	**16.7**	**7787**	**8368**	**8077**
	PAN 6479	84	87	86	3.9	4.1	4.0	8044	9617	8830
	PAN 6480	65	72	69	7.7	6.6	7.2	8961	9100	9031
	CRN 3584	81	70	76	3.6	3.0	3.3	8254	8565	8410
	MEAN	**77**	**76**	**77**	**5.1**	**4.6**	**4.8**	**8420**	**9094**	**8757**
	TRIAL MEAN	68	65	66	17.6	14.8	16.2	7931	8326	8129
	LSD (P<0.05)	NS	NS	9	NS	NS	6.5	743	743	525
92/93	PAN 6552	109	90	100	1.9	19.7	10.8	4271	5239	4755
	RS 5206	110	90	100	2,2	19.8	11.0	4380	5113	4747
	PAN 6528	108	98	98	1.8	18.3	10.1	4684	5491	5089
	MEAN	**109**	**89**	**99**	**2.0**	**19.3**	**10.6**	**4445**	**5281**	**4864**
	SNK 2950	108	90	99	2.0	17.3	9.6	4756	5309	5033
	PAN 6364	104	86	95	2.2	16.7	9.4	4603	5212	4908
	SNK 2888	107	88	98	1.9	19.0	10.4	5113	6020	5567
	MEAN	**106**	**88**	**97**	**2.0**	**17.7**	**9.8**	**4824**	**5514**	5169
	PAN 6479	124	94	109	0.6	7.5	4.1	4679	5931	5305
	PAN 6480	118	93	106	0.9	10.0	5.4	5435	6704	6069
	CRN 3584	115	94	105	0.9	7.4	4.2	5042	6656	5849
	MEAN	**119**	**94**	**107**	**0.8**	**8.3**	**4.6**	**5052**	**6430**	**5741**
	TRIAL MEAN	112	90	101	1.6	15.1	8.3	4774	5742	5258
	LSD (P<0.05)	NS	NS	4,9	3.4	3.4	2.4	NS	NS	650
93/94	PAN 6552	75	74	74	28.3	36.4	32.4	4656	4303	4479
	RS 5206	73	80	76	39.4	39.4	35.5	4257	3566	3912
	PAN 6528	80	78	79	24.5	28.9	26.7	3816	3031	3423
	MEAN	**76**	**77**	**76**	**30.7**	**34.9**	**31.5**	**4243**	**3633**	3938
	PAN 2950	75	70	73	30.0	32.9	31.5	4824	4661	4743
	PAN 6364	76	74	75	31.7	31.7	31.2	4985	5027	5006
	PAN 2888	74	73	73	33.4	33.4	30.1	5854	5112	5483
	MEAN	**75**	**72**	**74**	**31.7**	**32.7**	**30.9**	**5221**	**4933**	**5077**
	PAN 6479	84	79	81	11.9	19.5	15.7	6003	5563	5783
	PAN 6480	79	74	77	11.0	21.0	16.0	6620	5924	6272
	CRN 3584	83	73	78	13.6	15.1	14.3	5647	5687	5667
	MEAN	**82**	**75**	**79**	**12.2**	**18.5**	**15.3**	**6090**	**5725**	5907
	TRIAL MEAN	78	75	76	23.2	28.7	25.2	5185	4764	4974
	LSD (P<0.05)	4,3	4,3	3,1	N S	N S	8.2	N S	N S	720

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[a] DAP is days after planting; estimated from logistic model (Vanderplank, 1963)

[b] Area under disease progress curve (AUDPC), standardised by dividing AUDPC value by time duration of epidemic

[c] Tillage - "Conv" is clean cultivation

Table 5. Phenotypic [(a)] and genotypic correlations among grey leaf spot disease ratings and grain yield at different locations, seasons and tillage systems.

Correlation Analysis				
Location	**Season**	**Tillage**	**Correlation (r)**	
			Phenotypic	**Genotypic**
Cedara	1991/92	Conventional	0.1239	0.1369
Cedara	1991/92	Stubble	-0.6361**(b)	-1.0000**
Cedara	1992/93	Conventional	-0.1922	-0.4775**
Cedara	1992/93	Stubble	-0.3435*	-0.6161**
Cedara	1993/94	Conventional	-0.6452	-0.7072**
Cedara	1993/94	Stubble	-0.6859**	-0.6902**
Greytown	1991/92	Conventional	0.0730	0.0000
Greytown	1992/93	Conventional	0.0278	0.1119
Greytown	1993/94	Conventional	-0.5236**	-0.6354**

(a) Phenotypic data were obtained from field assessment of disease.

(b)** Correlation highly significant ($P<0.01$)

* Correlation significant ($P<0.05$)

Discussion

Grey leaf spot is the most yield limiting disease of maize in KwaZulu-Natal. No commercial hybrids are resistant to the disease in South Africa, but groups of hybrids were found to have different levels of susceptibility.

Disease severity is expected to be higher under stubble than conventional tillage, and in the United States GLS is often associated with stubble tillage systems. In contrast, no differences were found in this study in disease levels between stubble and conventional tillage treatments in seasons with normal or above average rainfall. Where GLS is established, the disease is a problem under favourable weather conditions in both stubble and conventional tillage. In a dry season, disease may infect maize earlier under stubble, but its subsequent slow development and effect on yield is offset by improved yield from the beneficial effects of soil moisture retention under stubble than conventional tillage. This is important in South Africa, which frequently experiences low and erratic rainfall and where soil and moisture conservation are the key to sustainable crop production. In all seasons, hybrid groups less-susceptible to GLS will have an advantage, as disease develops in these groups later than hybrids more susceptible to GLS (Table 4).

Linear regression models used in this study consistently showed that hybrid groups less-susceptible to GLS, with lower GLS levels, had higher grain yields than hybrids more susceptible to GLS. This suggests that less-susceptible hybrid groups have some form of partial resistance to GLS. This is illustrated by PAN 6480, which yielded consistently higher than predicted (Figure 1). The hybrid groups susceptible to GLS, with higher than average GLS levels, varied in their predicted responses. PAN 6552 had relative yields higher than predicted, is very susceptible, but has some tolerance to GLS. PAN 6528, on the other hand, had lower than predicted yields, indicating that this hybrid is inherently susceptible to GLS. Hybrid groups with intermediate susceptibility and average GLS levels had predicted or higher than predicted relative yield responses. SNK 2888, with relative yields higher than the trial mean (100%), had higher than predicted yield responses, indicating this hybrid to have greater tolerance to GLS. Tolerance is defined as the ability of plants to produce a good crop even when they are infected with a pathogen (Agrios, 1988). The ability of linear regression models to differentiate maize hybrids by relative yields and disease levels into less-susceptible (partially resistant), susceptible and tolerant categories, shows that the linear regression is a useful technique in the selection of hybrids for areas where GLS is a problem. These results support the approach of Stromberg and Donahue (1986).

The development of Gupta's Bestest method of ranking hybrids into subsets for disease susceptibility and yield performance (Table 1) supported results obtained from linear regression analysis. This is a useful method to establish groups of hybrids that are least susceptible to disease and subsets that have the highest grain yields. The method, however, is unable to distinguish hybrids that may be tolerant of disease. Gupta's test was favoured over other multiple comparisons such as Tukeys test, which is conservative when more than 20 treatments are present.

The logistic model (Vanderplank, 1963) was used to calculate DAP to 1% leaf-blighting. Grey leaf spot reached 1% blighting earlier in susceptible than less-susceptible hybrids. The earlier appearance of GLS with correspondingly higher levels of disease (AUDPC) in susceptible hybrids exposes this group to greater risk from GLS, as they are subjected to blighting over a longer period than hybrids that are less-susceptible to GLS. This may be of importance in areas where fungicides are needed for the control of GLS. The earlier appearance of disease in susceptible hybrids may require more spray applications for control than less-susceptible hybrids. The model may be useful in providing added data for selection of hybrids in areas subject to GLS, and making decisions about spraying requirements of each cultivar.

Grey leaf spot, previously restricted to the province of KwaZulu-Natal, is increasing in its distribution and severity in South Africa and neighbouring countries. Its increase in prevalence and severity indicates the disease to have the potential to be a limiting factor in these important maize-producing areas of southern Africa. The selection of less-susceptible or tolerant hybrids in these areas will reduce the risk from the disease and ensure more consistent grain and silage production.

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CHAPTER 3

MANAGEMENT PRACTICES TO REDUCE GRAY LEAF SPOT OF MAIZE(1)3

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Abbreviations: AUDPC, area under disease progress curve; DAP, days after planting; GLS, gray leaf spot; LSD, least significant difference.

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ABSTRACT

The beneficial effects of stubble tillage on soil and water conservation are widely recognised, but surface stubble also increases the potential for crop damage by fungal pathogens that over-winter in the previous season's debris. In recent years gray leaf spot (GLS) has become a major yield- limiting disease, resulting in grain yield losses as high as 60% in high yield-potential maize production areas. A study was launched to investigate strategies that could be adopted to facilitate the continuation of conservation tillage practices without exposing maize to unnecessarily high risk from GLS. The aim of the study was to investigate the interactive effects of four tillage practices leaving varying amounts of surface residues and fungicide treatments for the control of stubble-associated pathogens. Tillage treatments were no-till, chisel, chisel and disc, and plowed tillage practices. In the hot, dry season, of 1994/95, which was unfavorable for GLS, the onset of disease was 23 days earlier in no-till, which had a higher disease incidence than conventional tillage. The benefits of conserved soil moisture under stubble tillage with concomitant higher grain yields than conventional tillage in the hot, dry season offset the detrimental effects of higher disease. In the 1993/94 season which was favorable for GLS, there were no differences in disease between tillage treatments. Results from the study indicate that plowing aimed at reducing surface stubble is unlikely to be successful as a practice to manage GLS in KwaZulu-Natal, where there is a high incidence and severity of the disease. Conventionally plowed treatments across the mean of four seasons showed no yield advantages over stubble tillage treatments. Over the four seasons of the study, grain yield responses to fungicide treatment ranged

from 477 kg ha^{-1} in unfavorable seasons to 3 830 kg ha^{-1} in seasons favorable for GLS. The judicious application of fungicides will reduce the risk of financial loss from GLS and will allow the continuation of the desirable stubble tillage practice in sustainable farming systems.

INTRODUCTION

Gray leaf spot (GLS) disease of maize (*Zea mays L.*), caused by the fungus *Cercospora zeae maydis* Tehon and E.Y. Daniels, was first observed in KwaZulu-Natal in 1988. The disease was initially confined to the more humid mistbelt but has since spread rapidly to infect most of the maize in the province. It has also been identified in neighbouring provinces (Ward and Nowell, 1997). Research conducted at Cedara Agricultural Development Institute, Cedara, near Pietermaritzburg, South Africa, has shown the disease to be capable of reducing grain yields by 30 to 60%, depending on hybrid susceptibility and favorable weather conditions. The disease has posed a serious threat to economic production of maize (Ward, *et al.*, 1994). Maize is the only known host of *C. zeae-maydis* and the pathogen overwinters only in infected maize debris (Beckman and Payne, 1982; Latterell and Rossi, 1983). The increase in incidence and prevalence of GLS has been linked with continuous maize production (Anderson, 1995; Beckman and Payne, 1982; Latterell and Rossi, 1983) and conservation tillage practices that leave maize residues on the soil surface (Payne, Duncan and Adkins, 1987; Rupe, Siegel and Hartman, 1982; Stromberg and Donahue, 1986). Disease levels increase with the amount of residue on the soil surface (de Nazareno *et al.*, 1993). It is the fungus within the infected debris from the previous season that produces conidia, following long periods of warm, humid weather. These conidia infect the lower leaves of the next maize crop (Beckman and Payne, 1982). Following conditions favorable for disease, lesions that develop on the lower leaves produce conidia that serve as inoculum for secondary infection on upper leaves (Stromberg, 1986).

Whilst the beneficial effects of stubble tillage on soil and water conservation are widely recognised, these benefits are frequently offset by the increased potential for crop damage by fungal pathogens that overwinter in the previous season's debris (Anderson, 1995; de Nazareno *et al.*, 1993). In recent years in South Africa, stubble-related diseases have become major obstacles to the promotion of conservation tillage. This is well illustrated by the severe ear rot epidemic of the 1986/87 season, which resulted in extreme financial losses to maize farmers. These losses were ascribed to the fungal pathogen build-up in maize debris associated with an increase in conservation tillage (Mallett and Berry, 1991). Farmers were officially advised to discontinue stubble practices and to plow under or, in cases of severe disease pressure, to burn the previous season's crop residues (Berry, pers. comm., 1995(1)). Crop rotations were also suggested as an alternative control measure. However, preliminary investigations suggested that where maize residue loads were high, as is the case at Cedara, a single crop of soybeans would not guarantee ear rot protection when stubble tillage was practiced (Mallett and Berry, 1991).

Gray leaf spot has become a major yield-limiting disease of maize in the United States (Ringer and Grybauskas, 1995), and the disease spread finds a parallel in South Africa where the pathogen is moving to areas of lower humidity in the provinces of KwaZulu-Natal and Mpumalanga, as well as to neighboring countries. No commercial maize cultivars have been found to be resistant to GLS, but research at Cedara has identified high-yielding hybrids which are less susceptible to the disease (Ward and Nowell, 1997). Other research has shown that fungicides provide effective control of GLS of maize grown under stubble tillage (Ward *et al.*, 1994). At current costs, the break-even yield for fungicide and its application allows for economical use of fungicides in commercial maize in South Africa. The ability to control these residue-borne diseases has opened the way for a return to the desirable practice of conservation tillage.

The aim of the present study was to investigate the interactive effects of tillage systems and fungicide treatments in reducing fungal diseases that mitigate against conservation tillage practices.

MATERIALS AND METHODS

Experimental design and treatments

The study was conducted at Cedara Agricultural Development Institute (CADI) at Cedara (29°31'S, 30°18'E, and altitude 1070 m). The site was north-facing, gently-sloping and comprised well-drained, deep sandy-clay loam soils of the Hutton form and Doveton series (MacVicar, 1991). Maize had been grown on the site since 1977, and in 1983 a trial comparing a range of tillage treatments was initiated (Berry *et al.*, 1985). Four treatments, no-till, chisel, chisel and disc and conventional plowed, leaving stubble residues of 82%, 41%, 26% and 3%, respectively, on the soil surface, were included in a randomized complete blocks design with four replicates (Table 1). Each plot comprised twelve, 60 m rows, spaced 0.75 m apart. Since GLS was observed, the tillage treatments were split for fungicide sprayed and unsprayed sub-treatments, from 1993/94 until the conclusion of the study. Each fungicide sub-treatment comprised twelve, 30 m rows.

To limit the spread of inoculum from plot to plot, the least susceptible commercial hybrids to GLS, were planted throughout the trial. The widely grown commercial hybrids, PAN 6480 in 1992/93, and PAN 6479 in subsequent seasons, were planted in the trial, and only the central two-rows of the 12 rows of each plot were assessed for GLS and harvested.

Tillage practices

Four tillage practices were evaluated: (1) no-till planting into the previous season's corn residue; (2) chisel plowed to a depth of 120 mm, once in winter and again immediately prior to planting. Chisel-tines were fitted with sweeps and spaced 310 mm apart; (3) chisel plowed to 120 mm in winter, and disced to 150 mm depth with an offset disc prior to planting; and (4) disced followed by mouldboard plowing in early spring and disced again immediately prior to planting. Percentage maize stubble was determined at planting using a siting frame described by Lang and Mallett (1982). Fertilizer was band-applied to provide 32 kg N, 48 kg P, 63 kg K and 2.4 kg Zn ha^{-1} when the maize was knee-high. Standard weed and insect-pest-control practices for the area were followed. The trial was machine planted at a population of 50 000 seeds ha^{-1} in early November each season and the final plant stand was approximately 44 000 plants ha^{-1}. Fungicide treatments were applied from the 1993/94 season. Initial sprays were initiated when GLS symptoms were present on the basal three to five leaves of corn plants and a second application followed 20 to 27 days later. In 1993/94 the fungicide was applied 81 and 101 days after planting (DAP), in 1994/95, 75 and 98 DAP and in 1995/96, 75 and 101 DAP. Spray solutions were applied to sprayed plots using a backpack sprayer fitted with an overhead boom on which three TK 2.5 floodjet nozzles were mounted 500 mm apart. Spray solutions contained 187.50 g carbendazim plus 93.75 g flusilazole in 60 L water ha^{-1} (Punch Xtra, Du Pont de Nemoirs and Company).

Table 1. **Tillage treatments and tillage operations leaving varying levels of stubble cover on the soil surface at planting**

Tillage treatment	Tillage operations, time of application and working depths	% stubble cover+
No-till	Soil disturbed only during planting.	82
Chisel	Chisel plowed to 120 mm-depth, once in winter and again just prior to planting. The chisel plow had a line spacing of 310 mm and was fitted with sweeps.	41
Chisel & disc	Chisel plowed once to 120 mm-depth in winter (same implement and settings as for the chisel treatment), and disced to 150 mm with an offset disc just prior to planting.	26
Plowed	Disced once to 150-mm depth with an offset disc, mouldboard plowed in September to 250-mm depth, and disced once to 150-mm just prior to planting.	3

\+ Mean per cent cover over the trial area, estimated using siting fram described by Lang and Mallett (1982)

Disease assessment

The two central rows, 20 m long in each plot, were assessed at regular intervals for disease. Percentage disease was assessed visually using standard whole-plant area diagrams developed and described by Ward *et al.* (1996). These data were used to calculate area under disease progress curve (AUDPC). The AUDPC is a quantitative summary of the disease epidemic and is based on a trapezoidal integration program (Berger, 1981). The AUDPC was standardized (SAUDPC) by dividing the AUDPC value by the duration of the epidemic to allow disease comparisons from one season to the next. Percentage disease data were transformed to fit the logistic model described by Vanderplank (1963) to calculate infection rates. The fitted regression functions of the transformed values were used to estimate the onset of 1% disease in terms of DAP (first signs of disease). The harvested area was the central two, 20 m rows and grain yields are reported at a moisture content of 12.5%.

Statistical analysis

SAUDPC, final disease severity, DAP to onset of 1% disease, infection rates (r x 100), grain yields and percent lodged maize for individual seasons were processed by analysis of variance (ANOVA) using Genstat 5.2 (Anon., 1987). The level of significance for treatment differences were considered significant when probability of greater F-values were 0.05. Mean separations were based on the LSD at the 5% level of probability. Bartlett's χ^2 test was used to establish homogeneity of variances (Gomez and Gomez, 1984). Where the test indicated variances to be homogeneous a combined analysis of data across seasons was conducted. This was conducted on SAUDPC and grain yield data.

RESULTS AND DISCUSSION

Disease development

Weather conditions favorable for disease development varied over the seasons of the study (Table 2). The 1992/93 season was unfavorable for disease, especially during the vegetative growth stages. Conditions during the growing season were dry, and only 67% of the mean rainfall was recorded. The 1993/94 season was favorable for disease, with above-average well-distributed rainfall and warm temperatures. In contrast, 1994/95 was hot and dry, with only 66% of the mean rainfall and higher than the 50 year mean temperature during the vegetative growth stages, and was unfavorable for GLS. The 1995/96 season experienced excessive rainfall that was 30% higher than the 50 year mean, but relatively low temperatures, lower than the 50 year mean, in December, early January and in March, were unfavorable for disease development.

Unsprayed treatments

Tests for homogeneity of variance of disease (SAUDPC) using Bartlett's χ^2 over the four seasons showed variances to be homogeneous (χ^2 = 6.76, NS). The combined analysis indicated more disease (SAUDPC = 28.3, Table 3b) during the favorable 1993/94 season than in other seasons. Final disease severity in 1993/94 was high (93.4%, Table 3a), 123 DAP or 22 days before physiological maturity. There were no differences in disease (SAUDPC) or final disease severity between tillage treatments. In the seasons less favorable for GLS and with less disease (SAUDPC) than 1993/94, final disease severities close to physiological maturity were only 38.9%, 31.4% and 34.4% in the 1992/93, 1994/95 and

Table 2. **Rainfall and temperatures at Cedara for the maize growing seasons 1992 to 1996.**

	Oct	Nov	Dec	Jan	Feb	Mar	Apr	Total
Rainfall (mm)								
1992/93	34	83	69	69	108	115	25	503
1993/94	133	63	162	206	127	113	37	846
1994/95	69	21	131	62	20	130	62	495
1995/96	95	110	303	147	170	140	18	983
Mean (50 year)	125	116	126	158	130	103	32	789
Mean temperature °C								
1992/93	17.7	18.5	20.3	21.0	20.0	19.4	17.3	
1993/94	17.0	18.3	19.4	19.9	19.4	19.0	17.1	
1994/95	15.0	19.0	20.2	20.8	22.3	19.1	15.6	
1995/96	16.9	18.5	17.9	20.3	20.2	18.4	15.6	
Mean (50 year)	17.1	18.4	19.9	20.6	20.4	19.2	17.6	

1995/96 seasons, respectively. In the 1992/93 and 1995/96 seasons, there was lower overall disease and final disease severity in conventional than in no-till treatments.

Table 3. The effect of four tillage treatments and fungicide sprays on final disease severity and SAUDPC of gray leaf spot infected maize

	Season							
a) Final disease severity at:	140 DAP† 1992/93	123 DAP 1993/94		140 DAP 1994/95		140 DAP 1995/96		
Tillage	Unspr†	Spr	Unspr	Spr	Unspr	Spr	Unspr	
No-till	58.7 a ‡	11.3	95.6	1.4	48.8	15.6 a	46.2 c	
Chisel	35.0 b		93.8	1.5	27.5	10.0 b	33.8 d	
Chisel & disc	36.2 b	8.1	93.8	1.3	25.6	9.4 b	30.6 de	
Conventional	25.6 c	7.5	92.5	1.3	23.8	8.6 b	26.9 e	
		8.8						
Mean	8.30	NS		NS				
LSD	38.9	8.9 f*		1.3 f	31.4	10.9 f	34.4 g	
CV%	13.3	3.1		61.4		9.3		
b) SAUDPC§	Unspr†	Spr	Unspr	Spr	Unspr	Spr	Unspr	Mean (unspr)
No-till	24.7 a‡	6.1	30.6	1.3	16.0 a	7.2 a	17.6 c	22.2 a
Chisel	12.6 b	5.2	29.5	0.8	9.8 b	5.1 b	14.9 d	16.6 b
Chisel & disc	10.9 b	4.7	29.3	0.6	8.3 c	5.0 b	12.9 de	15.4 b
Conventional	9.1 b	4.8	24.0	0.7	6.6 c	5.1 b	11.4 e	12.8 b
Mean	14.3	5.2 e*	28.3 f	0.8 e	10.2 f	5.6 e	14.1 f	16.7
LSD	5.23	NS		4.01		2.1		
CV%	22.8	13.4		47.4		10.5		
Unsprayed mean	14.3 h		28.3 g		10.2 i			16.7
LSD	2.49							4.01

† Abbreviations: DAP, days after planting; Spr, fungicide sprayed; Unspr, unsprayed; LSD, least significant difference; CV% coefficient of variation per cent.

‡ Treatment means within columns with a letter in common are not significantly different at the 0.05 probability level by LSD method; NS, not significant.

§ SAUDPC is the area under disease progress curve, standardised the dividing the AUDPC value by the duration of the epidemic.

* Sprayed and unsprayed treatment means within rows within seasons, with a letter in common are not significantly different at the $P<0.05$ level of probability.

The effect of tillage practices on GLS development over the four seasons was dependent on prevailing weather conditions and the amount of stubble remaining on the soil surface following tillage treatments. In seasons unfavorable for disease, GLS was observed 38, 12 and 13 days earlier in no-till (with a higher stubble load) than conventional treatments, (with little stubble) in the 1992/93, 1994/95 and 1995/96 seasons, respectively (Table 4a). In 1993/94, favorable for GLS, there were no differences between tillage treatments to onset of 1% disease. This indicated, in unfavorable seasons, that the earlier onset of disease in no-till was associated with the stubble present on the soil surface, and this was not the case in favorable seasons. The low infection rate (r) in unfavorable seasons resulted in lower disease (SAUDPC) than in the 1993/94 season, favorable for GLS. The high level of leaf blighting (93.9%) 22 days before physiological maturity in 1993/94 was largely due to the high infection rate. The lower infection rates in unfavorable seasons resulted in lower levels of leaf-blighting at physiological maturity. It can be concluded from these results that surface stubble amounts on the soil surface has a lesser effect on disease development in the wet season favorable for GLS in areas where the disease is epidemic. This conclusion is substantiated by a significant interaction ($P < 0.05$) between year and tillage in the analysis of variance of SAUDPC.

Fungicide sprayed treatments

The application of fungicides resulted in less disease (SAUDPC), lower final disease severity, and lower infection rates than in the unsprayed treatment (Table 3a and b and Table 4b). Only in 1995/96 was more final disease present in no-till than other tillage treatments. The results indicate that fungicides are highly effective in controlling development of GLS.

Table 4. The effect of four tillage treatments and fungicide sprays on days to 1% onset of disease and infection rate of gray leaf spot infected maize

Tillage	Season						
	1992/93	1993/94		1994/95		1995/96	
	Unspr †		Unspr		Unspr		Unspr
a) **Days to 1% Disease**							
No-till	42 a ‡		61		88 a		82 a
Chisel	70 b		63		92 a		83 a
Chisel & disc	77 b		65		90 a		91 b
Disc & plow	80 b		67		111 b		95 c
Mean	67		64		95		88
LSD	20.5		NS		11.9		4.3
CV%	19.1		5.8		7.8		3.1
b) **Infection rate (r x 100) §**	**Unspr†**	**Spr**	**Unspr**	**Spr**	**Unspr**	**Spr**	**Unspr**
No-till	4.8	2.9	12.1	2.5	5.0	6.3	7.4
Chisel	5.2	2.8	12.2	2.6	6.0	5.9	6.8
Chisel & disc	7.7	3.4	13.1	3.5	5.5	5.8	6.7
Disc & plow	7.2	3.6	12.4	2.9	7.5	6.8	8.4
Mean	6.2	3.2 e	12.5 f	2.9 e	6.0 f	6.2 e	7.3 f
LSD	NS	NS		NS		NS	
CV%	27.7	6.1		31.4		14.9	

† Abbreviations: Spr, fungicide sprayed; Unspr, unsprayed; LSD, least significant difference; CV%, coefficient of variation percent

‡ Treatment and season means with a letter in common are not significantly different at the 0.05 probability level by the LSD method; NS, not significant.

§ Infection rate x 100, calculated by regressing the log-transformed data of disease on time and expressed as a percentage.

* Sprayed and unsprayed treatment means within seasons with a letter in common are not significantly different at the $P<0.05$ level of probability.

Grain yields

Unsprayed treatments

Variance in grain yields across four seasons were homogeneous (Bartlett's χ^2 = 7.32, NS). A significant interaction in the analysis of variance between season and tillage indicated that the tillage response was not consistent over all across seasons. The highest grain yield (7304 kg ha^{-1}) was obtained in the 1995/96 season, which had adequate rainfall for maize, but the relatively low temperatures reduced disease severity. The lowest grain yields (3679 kg ha^{-1}) occurred in 1993/94. This season had adequate rainfall for maize growth, but the moderate temperatures and rainfall favored high disease levels (93.9% leaf-blighting three weeks before physiological maturity). The pre-mature leaf-blighting was responsible for the decreased maize yields.

In the 1993/94 season, favorable for both GLS and high maize yields, no differences in final disease levels and amount of disease (SAUDPC) between tillage treatments were recorded (Table 3a and 3b). The crop was blighted well in advance of physiological maturity, and there were no differences in yield responses between conventional and other stubble tillage treatments, except for no-till, which had lower grain yields (Table 5a). In seasons less favorable for disease, such as 1994/95, with lower disease in conventional, and disc and chisel, than other tillage treatments, the conventional tillage (despite lower disease) had lower grain yields than other tillage systems that retained stubble on the soil surface (Table 5a). The higher grain yields under stubble are consistent with the findings by Berry *et al.* (1985), on the same site, that water deficiencies may occur during critical maize growth stages in dry years in conventionally plowed maize. The higher yield under stubble is due to soil water that is released to the crop over a longer period of time under stubble tillage systems. The lack of grain yield response in 1992/93, in spite of higher disease in no-till, may have been due, in part, to adverse conditions for disease, but was also due to lower initial inoculum. GLS was only observed at low levels for the first time in the previous season. The presence of GLS in the 1991/92 season was the reason for introducing fungicide treatments in subsequent seasons. The lack of yield response to tillage treatments in 1995/96 was due, in part, to low final disease severity, but also to adequate soil moisture. There was adequate moisture for plant growth and development and therefore no benefit from moisture-conserving tillage systems.

Table 5. The effect of four tillage treatments and fungicide sprays on grain yields and stem lodging of gray leaf spot infected maize

	Season							Mean
Tillage	1992/93	1993/94		1994/95		1995/96		
	Unspr †	Spr	Unspr	Spr	Unspr	Spr	Unspr	Unspr
a) Grain yields								
No-till	5569	6586 a ‡	3003 a	5751 a	5853 a	8527	6994	5355
Chisel	5907	7994 b	3624 b	5950 a	5352 a	8710	7263	5536
Chisel & disc	5635	7995 b	3969 b	5798 a	5192 a	8775	7334	5533
Conventional	5525	7462 b	4120 b	4847 b	4038 b	8951	7624	5327
Mean	5659	7509 e ‡	3679 f	5586 e	5109 f	8741 e	7304 f	5438
LSD	NS	886.6		903.6		NS		NS
CV%	5.1	7.0		7.4		2.5		7.7
Unspr mean	5659 i	3679 g		5109 h		7304 j		
LSD	327.0							
b) Stem lodging %								
No-till	20	18 a	46 a	1	2	0.5	1.1	
Chisel	25	6 b	25 b	1	2	0.2	1.3	
Chisel & disc	31	10 a	19 b	3	3	0.3	0.9	
Conventional	28	12 a	16 b	2	2	0.3	0.9	
Mean	26	12 e	27 f	2	2	0.3 e	1.0 f	
LSD	NS	9.7		NS		NS		
CV%		44.9				81.0		

† Abbreviations: Spr, fungicide sprayed; Unspr, unsprayed; LSD, least significant difference; CV%, coefficient of variation percent

‡ Treatment and season means with a letter in common are not significantly different at the 0.05 probability level by the LSD method; NS, not significant

Fungicide sprayed treatments

The yield responses of tillage treatments to fungicide application in all seasons of treatment were consistent with the responses in unsprayed maize. Fungicide treatment, however, resulted in higher grain yields than unsprayed maize (Table 5a). The results confirm the effectiveness of fungicides in controlling GLS.

Stem lodging

There were no differences in the amount of stem lodging between tillage treatments over the seasons of the study, except in 1993/94, the season favorable for GLS (Table 5b). There was more stem lodging in no-till sprayed and unsprayed maize. There was also more lodging in unsprayed maize, which again confirms the beneficial effects of fungicides in controlling GLS. It is widely accepted that GLS pre-disposes maize to lodging (Stromberg, 1986). This was especially evident in 1993/94, when high disease severity caused severe blighting of plants three weeks before physiological maturity. Fungicide treatment in this season and in 1995/96 effectively reduced the amount of lodging.

Economics of fungicide treatment

The 1995/96 maize price paid to producers averages \$144 ton^{-1} and present costs for fungicides and aerial spray charges are \$30 ha^{-1}. The break-even yield to cover chemical and aerial spray costs is a gain of approximately 210 kg grain ha^{-1} per fungicide application. In seasons of high disease, such as 1993/94, the gain in yield from fungicide treatment far exceeded the break-even.

In seasons of low disease, such as 1994/95, the gain in yield from fungicides again exceeded the break-even in all tillage treatments. The judicious use of fungicides is therefore economical, even in years of low disease, in all tillage treatments except no-till.

CONCLUSIONS

The application of fungicides to maize grown under four tillage practices reduced pathogen infection rates, disease (SAUDPC) and final disease severity, and, in most seasons are more likely to be effective in managing GLS than tillage practices aimed at the reduction of initial inoculum.

In seasons unfavorable for GLS, onset of disease may be earlier in stubble treatments than conventional tillage and, in the hot, dry season, conventional tillage aimed at the reduction of initial inoculum may be more effective than no-till in reducing disease in unsprayed maize. However, the benefits of improved soil moisture conservation under stubble tillage systems compensate for yield losses that might be expected from increased disease. In the cool, moist season, disease development is restricted by lower temperatures to limit the extent of leaf blighting at physiological maturity and reduction of grain yields. In seasons favorable for GLS, which are more frequent in KwaZulu-Natal, there were no differences among tillage systems to onset of disease. Infection rates are high and severe leaf-blighting results in all tillage systems before the crop is physiologically mature, and grain yields are reduced significantly. Under these conditions, of adequate rainfall and temperature, large grain yield benefits can be expected from fungicide treatment under all tillage practices.

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CHAPTER 4

[4]FUNGICIDE RESPONSES OF MAIZE HYBRIDS TO GREY LEAF SPOT DISEASE

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Abstract

Key words: grey leaf spot, fungicide responses, Maize hybrids

Grey leaf spot disease of maize (*Cercospora zeae-maydis*) has seriously decreased grain yields in the province of KwaZulu-Natal, South Africa, and has spread to infect maize in neighbouring provinces. No commercial hybrids resistant to the disease have so far been identified, and fungicides have been shown to reduce disease severity. The response of sixty-four commercial hybrids to grey leaf spot under fungicide treatment were studied over two seasons. Regression analyses was conducted to examine the effects of grey leaf spot of fungicide-sprayed disease severity against unsprayed maize disease severity and, gain in yield due to fungicide treatment against unsprayed disease severity. Overall, fungicides reduced disease severity and the linear regression of gain in yield against disease severity enables the identification of hybrids with optimum responses to fungicides. Under low disease levels hybrids responded less to fungicides than under high disease levels. NS 9100 and PAN 6480 were least susceptible, SNK 2888, RS 5206 and RS 5232 were most susceptible, whilst PAN 6479 and PAN 6549 were of intermediate susceptibility to GLS. The most susceptible hybrids had the highest responses in control of leaf-blighting and gain in yield. Hybrids that had less leaf-blighting than predicted from the regression graph also yielded higher than predicted on the graphs indicating these hybrids to be less susceptible to grey leaf spot. These less susceptible hybrids are likely to require fewer fungicide treatments than more susceptible hybrids and are at

lesser risk of serious yield losses.

Abbreviations: GLS, grey leaf spot., AUDPC, area under disease progress curve

Introduction

Grey leaf spot (GLS) disease of maize (*Zea mays* L.), caused by the fungus *Cercospora zeae-maydis* Tehon and E.Y. Daniels, is a relatively new disease in South Africa. It is capable of reducing maize grain yields by as much as 30 to 60 percent and reduces the yield and quality of maize grown for silage (Ward & Nowell, 1997). Yield losses tend to be more severe with monoculture maize (Beckman & Payne, 1983; Latterell & Rossi, 1983), and conservation tillage practices that retain the previous season's infected maize residue on the soil surface (Rupe, Siegel & Hartman, 1982; Stromberg & Donahue, 1986; Payne, Duncan & Adkins, 1987). GLS may be managed through tillage practices that completely bury infested maize debris. (Payne & Waldron, 1983; Huff, Ayres & Hill, 1988). However, in the United States, the disease has recently been observed to move from reduced tillage situations to become a problem in fields where traditional conventional tillage practices are used (Perkins, Smith, Kinsey & Dowden, 1995).

In South Africa, maize is grown under a system of monoculture (Channon & Farina, 1991) and genetic resistance to GLS is likely to offer the long-term solution to the management of the disease. No commercial hybrids are resistant to the disease, and because alternative measures of control such as rotations and tillage practices have limited effects, fungicides are the main option for control of the disease (Ward, Laing & Nowell, 1997). At the current maize prices of R450 per ton of grain, the break-even yield for fungicide and its application per treatment is 290 kg of grain ha^{-1}. The use of fungicides in commercial maize production in South Africa would appear to be economic.

Fungicide sprays protect the upper leaves of the maize plant from disease until the crop is physiologically mature. Research at Cedara has shown that the effective period of control will vary between 29 and 32 days, if fungicides are applied when disease levels are between one and two per cent of leaf area infected (Ward, Laing & Rijkenberg, 1997). With early onset of disease more than one fungicide application is necessary to provide protection from disease until the crop is physiologically mature. Ward, J.M.J., Nowell, D.C., Laing, M.D. and Whitwell, M.I. (unpublished results) found that the onset of disease is later with hybrids least susceptible to GLS. Fewer fungicide treatments are required for these hybrids than those that are more susceptible to the pathogen (Ward, Laing & Rijkenberg, 1997).

The purpose of this study was to evaluate the response of commercially-grown maize hybrids to fungicide treatment, and to identify those hybrids which have optimum responses to fungicide application.

Materials and methods

The trials were conducted at the Cedara Agricultural Development Institute, Cedara (29°31'S, 30°17'E and alt 1 070 m), South Africa. Maize has been continuously grown at Cedara since the National Maize Hybrid Cultivar Trials commenced in 1982. Hybrids were evaluated for response to fungicide treatment during the 1992/93 and 1993/94 growing seasons. The experiments comprised 49 hybrids laid out in a 7 x 7 triple lattice design under stubble tillage. The experiment was replicated for fungicide sprayed and unsprayed treatments. The site was gently sloping and soils were well-drained, sandy-clay loams of the Hutton Form and Doveton Series (MacVicar, 1991). The trial was part of the National Maize Hybrid Cultivar Trial Series and the land preparation, fertilization, planting, weed and pest control practices, plot sizes and harvest details are described in Chapter 2 "Maize hybrid response to grey leaf spot under two tillage systems in KwaZulu-Natal, South Africa (page 32).

The 1992/93 season was characterised by low rainfall and hot days. The weather conditions until anthesis and during early grainfill were hot and dry and it was only in mid- to late-grainfill from mid-February that rainfall normalised. In contrast, rainfall during the 1993/94 season was above average and well-distributed throughout the growing season. Mists were abundant, especially in January and February, 1994. Temperatures were slightly lower than average (Table 1).

Table 1 **Rainfall and temperature at Cedara for the 1992/93 and 1993/94 growing seasons**

	Oct	Nov	Dec	Jan	Feb	Mar	Apr	Total
Rainfall (mm)								
1992/93	34	83	69	69	108	115	25	503
1993/94	133	63	162	206	127	113	37	846
Mean monthly	125	116	126	158	130	103	32	789
Mean Temperature								
°C	17,7	18,5	20,3	21,0	20,0	19,4	17,3	19,2
1992/93	17,0	18,3	19,4	19,9	19,4	19,0	17,1	18,6
1993/94	17,1	18,4	19,9	20,6	20,4	19,2	17,6	19,0
Mean monthly								

Cultivars

Sixty-four commercially-available hybrids in the National Cultivar Phase II series of trials were studied in the 1992/93 and 1993/94 seasons.

Fungicide treatment

In both seasons fungicide treatments commenced at first signs of disease : 76 days after planting (DAP) in 1992/93 and 74 DAP in the 1993/94 seasons. Three applications were made in each season at approximately 17-day intervals. Benomyl fungicide was applied in the first season at a rate of 375 g of active ingredient (ai) ha^{-1} and in 1993/94 a combination of 187,50 g carbendazim and 93,75 g flusilazole ai ha^{-1} was applied (Punch Xtra, Du Pont de Nemoirs and Coy). Spray solutions were applied with a CO$_2$ - pressurised back-pack sprayer fitted with a vertically mounted spray-boom having three Whirlrain ¼" WRW2-20° nozzles, spaced one metre apart. Full-cover sprays of 450 L ha^{-1} were applied to each maize row.

Disease and grain yield assessments

Whole-plant standard area diagrams described by Ward, Laing and Rijkenberg (1997) were used to in estimate percent disease severity. Assessments were made regularly on plants in the centre of each plot, commencing at first signs of disease until physiological maturity. These data were used in calculating the area under disease progress curve (AUDPC), which is an integrated summary of the disease epidemic. The AUDPC, calculated by the trapezoidal integration program (Berger, 1981), was standardised by dividing the AUDPC value by the duration of the epidemic. This was compared to the critical (single) point model of disease severity. As the correlations were highly significant, it was decided to use the critical point model as the disease index in the linear regression analysis. Grain yields were expressed in kg ha^{-1} at 12,5% moisture. Relative yield and disease data were obtained by dividing by the trial mean, and are expressed as a percentage. The relative disease is expressed similarly. The disease severities and yields have been presented on a relative basis to remove seasonal effects.

Regression analysis

A linear regression model was used to determine the yield responses and disease severity of GLS, and these were estimated by the linear regression model:

$$Y = Bo + Bi\ XI + Ei$$

where Y is the response variable (yield), Bo is the intercept (response when disease is zero), Bi is the slope of the regression line, XI is the regressor variable (disease severity at a particular stage) and Ei is the unexplained variation (error or residual). The regression analysis was conducted on Genstat 5.2 (Anon., 1987).

Results

Hybrids

Data from 64 hybrids, evaluated between one and two seasons, were used in the regression analyses.

Only 34 of the more commonly grown hybrids are listed in Table 2. Seven of these, evaluated for two seasons and representative of the main disease susceptibility groups, were selected for ease of presentation. PAN 6479 and PAN 6480 were least susceptible, SNK 2888, RS 5206 and RS 5232 were most susceptible, whilst NS 9100 and PAN 6549 were of intermediate susceptibility to GLS (Table 2).

Table 2. The mean relative disease severity and relative yields of 34 maize hybrids in response to fungicide treatment across the 1992/93 and 1993/94 seasons.

HYBRID		RELATIVE DISEASE SEVERITY	RELATIVE GRAIN YIELD	
			UNSPRAYED	SPRAYED
PAN	**6480**	**75**	**124**	**104**
SNK	2200	95	122	94
CRN	3584	55	121	101
SNK	2266	123	115	100
SNK	2154	77	113	100
PAN	**6479**	**54**	**113**	**98**
PAN	6034	110	113	117
SNK	2954	122	113	108
PAN	6363	66	112	100
TX	24	82	112	101
PAN	6496	97	109	107
SNK	**2888**	**123**	**109**	**101**
A	1598	91	106	100
PAN	6578	75	105	117
SNK	2042	88	105	94
SNK	2151	86	105	94
A	1556	89	104	95
SNK	2255	114	104	105
CRN	3816	89	104	95
SNK	2665	112	103	98
SNK	2246	103	101	91
PAN	6364	112	101	107
PHB	3412	83	101	103
SNK	2021	87	101	93
PAN	6146	106	99	120
NS	**9100**	**77**	**98**	**100**
SNK	2950	122	98	100
PAN	**6549**	**87**	**98**	**101**
CRN	4502	105	98	88
PAN	6552	136	93	108
RO	413	108	92	91
CRN	3414	118	89	-
CRN	4523	140	89	87
RO	410	160	88	104
A	1849	131	87	115
PHB	3442	87	87	102
CRN	4605	150	86	87
PAN	6140	112	86	121
RS	**5206**	**142**	**85**	**109**
RO	430	120	83	94
PAN	6528	126	82	106
CRN	4403	112	82	91
PHB	3427	106	78	94
SNK	2265	108	78	99
RS	**5232**	**149**	**76**	**95**

Disease severity

Disease severity varied between seasons. There was a lower mean disease severity of 31% in the 1992/93 season, compared to 82% in the 1993/94 season (Table 3).

Table 3 **The actual and predicted disease severity, grain yield and gain in yield (response) of unsprayed and fungicide sprayed maize hybrids in 1992/93 and 1993/94**

1992/93	Hybrid	Unsprayed		Sprayed			Gain in yields	
		Yield	Disease	Yield	Disease (%)		kg ha^{-1}	
		kg ha^{-1}	%	kg ha^{-1}	Actual	Predicted[a]	Actual	Predicted[b]
	PAN 6479	5931	14	6711	1,1	1,2	780	740
	PAN 6480	6704	18	7119	1,7	1,6	416	960
	NS 9100	5327	17	7294	1,4	1,4	1966	874
	PAN 6549	5722	20	7115	2,0	1,7	1393	1 050
	SNK 2888	6020	43	6689	3,7	3,7	669	2 301
	RS 5206	5113	53	7504	4,4	4,5	2292	2 837
	RS 5232	4678	58	6865	5,0	4,9	2186	3 105
	Trial Mean	5571	31	7075			1504	1 641
1993/94	PAN 6479	5563	52	9298	7,0	4,4	3735	2 751
	PAN 6480	5924	75	9770	4,2	6,4	3846	3 855
	NS 9100	4644	82	8802	3,3	7,0	4157	4 233
	PAN 6549	4286	90	9321	9,7	7,6	5035	4 631
	SNK 2888	5112	88	9839	10,0	7,5	4728	4 543
	RS 5206	3526	92	10259	10,0	7,8	6693	4 750
	RS 5232	3161	92	8458	14,2	7,8	5297	4 719
	Trial Mean	4629	82	9166			4536	4 376

[a] Predicted per cent disease calculated from regression analysis of fungicide-sprayed grey leaf spot disease severity against unsprayed disease severity.

[b] Predicted gain in yield calculated from regression analysis of gain in yield due to fungicide treatment against disease severity.

The regression of disease severity of fungicide-sprayed hybrids against disease severity of unsprayed hybrids accounted for 64,3% of the variance ($P < 0.001$). The slope of 0.08484 was highly significant ($P < 0.001$) (Fig. 1). At low disease levels experienced in 1992/93 the predicted disease severity of the sprayed maize was generally over-estimated (Table 3). Under the high disease levels experienced in 1993/94, the sprayed maize disease severities were much closer to those predicted. With the less susceptible hybrids, the actual disease severity of PAN 6479 was higher than predicted, and was lower than predicted for PAN 6480 (Fig. 1). Of the susceptible hybrids, RS 5232 had nearly double the disease severity than predicted, while RS 5206 and SNK 2888 also had higher than predicted levels of disease. The model predicted slightly higher disease severity for the intermediate susceptible PAN 6549, while NS 9100 had lower than the predicted disease level.

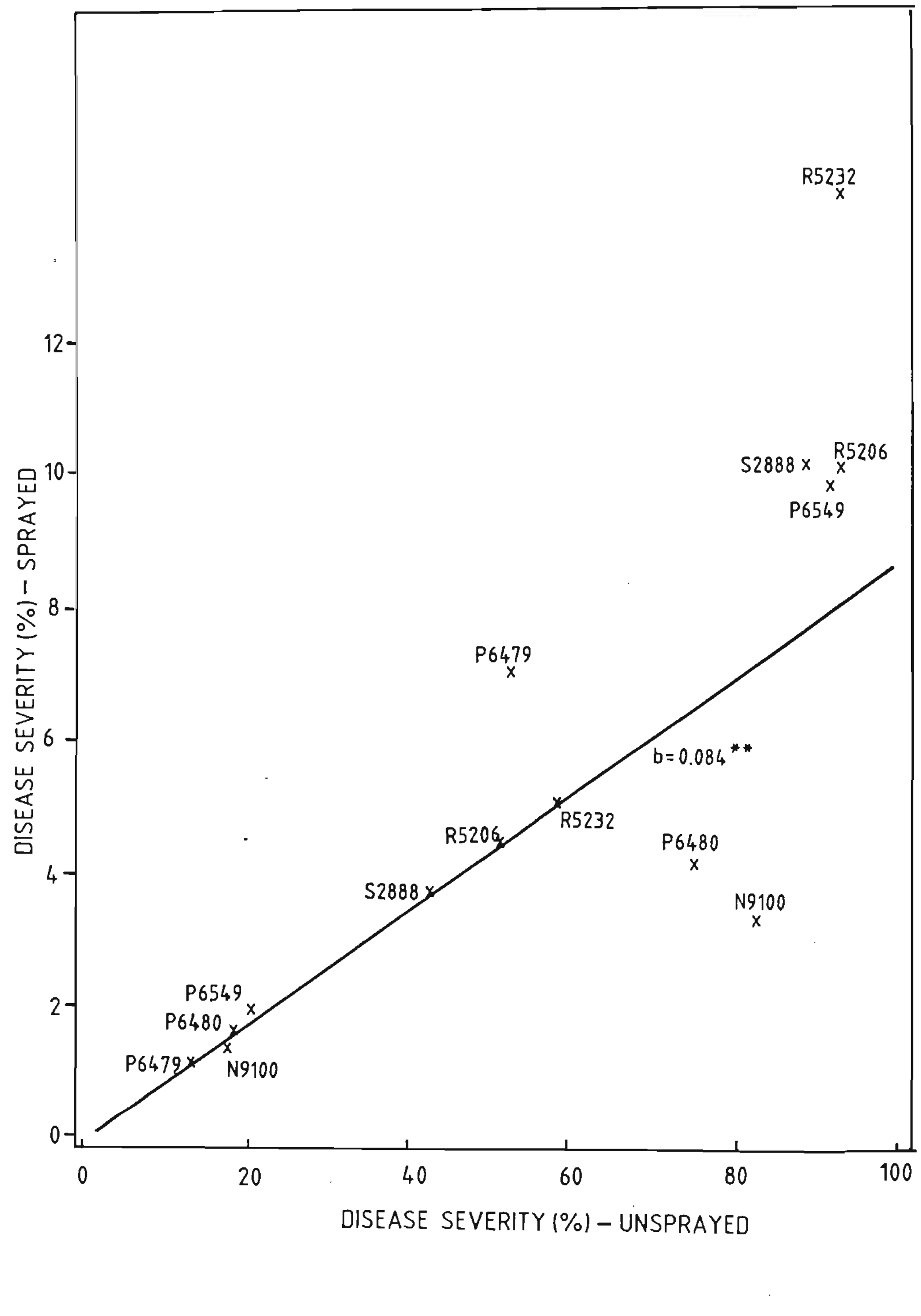

Figure 1. Regression analysis of fungicide-sprayed grey leaf spot disease severity against unsprayed disease severity at Cedara. Disease severity (expressed as a percent) of whole plants in central plot areas, was estimated at the milk stage of growth (R3 stage), 120 days after planting using standard whole plant diagrams. These regression analysis represent all hybrids evaluated over the 1992/93 and 1993/94 seasons. The means of only seven of the hybrids regressed are shown. N9100 and P6480 was least susceptible, R5206 and R5232 are most susceptible, S2888 is tolerant and P6549 and N6479 are intermediate in their reactions to grey leaf spot.

Grain yield and response to fungicide treatment

The overall grain yields and gain in yield due to fungicide treatment were lower in 1992/93 than in the 1993/94 season. In both seasons the hybrids with the lowest GLS levels, PAN 6479 and PAN 6480, had the highest unsprayed grain yields and the lowest responses to fungicide. In contrast, the most susceptible hybrids, RS 5206 and RS 5232 had the lowest unsprayed yields and the highest response to fungicides. SNK 2888, also with relatively high disease, had among the highest unsprayed yield. In 1992/93, the response of SNK 2888 to fungicides was relatively low (2301 kg ha^{-1}), whereas in 1993/94 the response was intermediate (4543 kg ha^{-1}) (Table 3).

When the gain in yield was regressed against disease severity, the linear regression accounted for 76% of the variation. Since the quadratic model only resulted in a 3.3% improvement, the linear model was retained. The intercept of the linear regression was -22,0 and the slope was 53,64, which was highly significant (Fig. 2). This model implies that with each percent increase in disease severity, there was a corresponding increase of 53,64 kg ha^{-1} response to fungicide treatment. Under the low disease levels in the drought of 1992/93 PAN 6480, SNK 2888, RS 5206 and RS 5232 responded less to fungicides than predicted. The response of PAN 6479 was within the 95% confidence limits, while NS 9100 and PAN 6549 responded better than predicted by the model. In the more humid 1993/94 season, highly conducive to GLS disease, the yield response of most of the seven hybrids was close to that predicted by the model (Table 3 and Fig. 2), except for RS 5206, which had the highest response to fungicides, of nearly 50% higher than predicted.

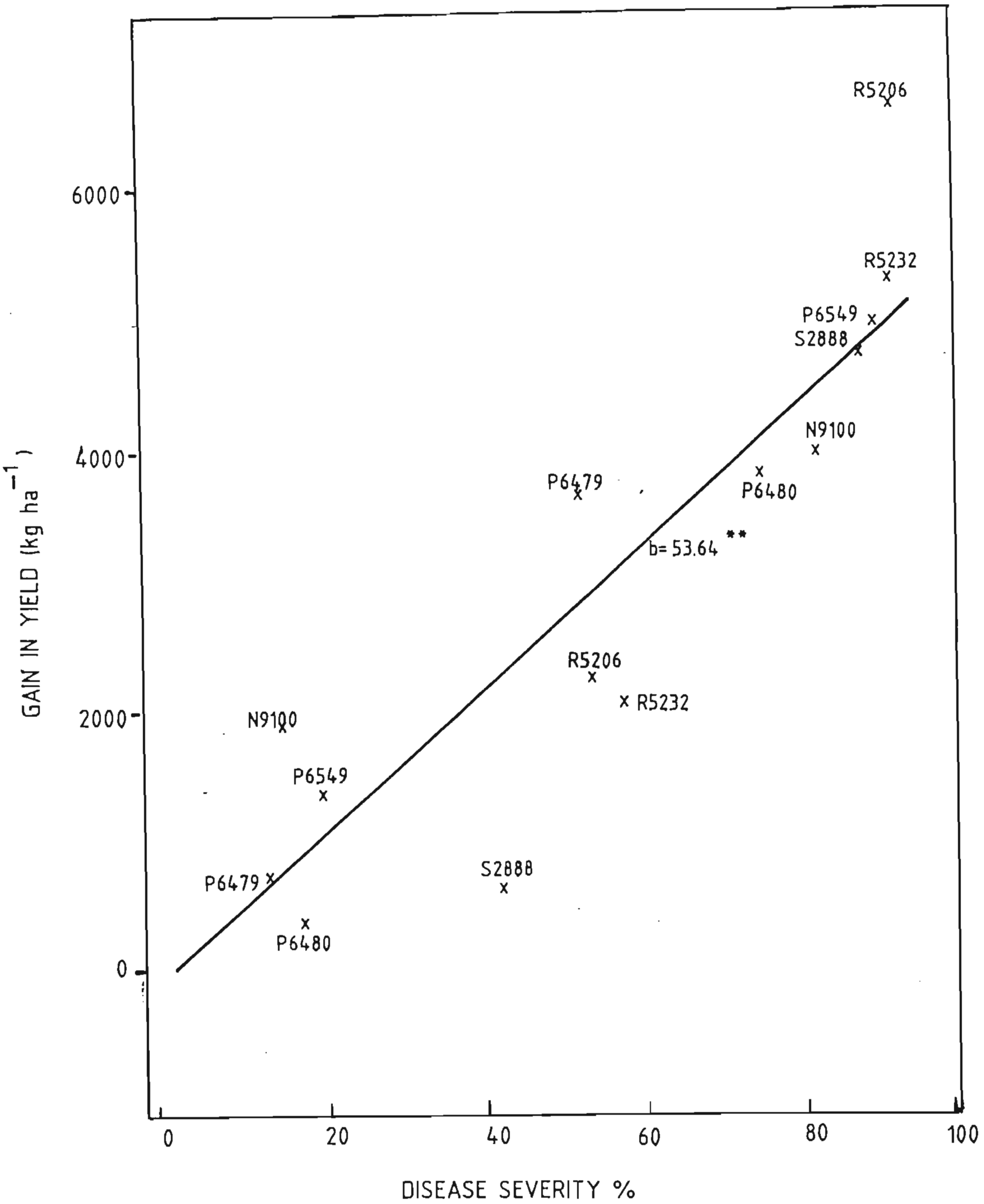

Figure 2. Regression analysis of gain in yield due to fungicide treatment against disease severity at Cedara. Disease severity (expressed as a percent) of whole plants in central plot areas, was estimated at the milk stage of growth (R3 stage), 120 days after planting using standard whole plant diagrams. The regression analysis represent all hybrids evaluated over the 1992/93 and 1993/94 seasons. The means of only seven of the hybrids regressed are shown. N9100 and P6480 was least susceptible, R5206 and R5232 are most susceptible, S2888 is tolerant and P6549 and P6479 are intermediate in their reactions to grey leaf spot.

Discussion

The regression analysis of the effect of disease severity on the response of maize hybrids to fungicide treatment may serve as a basis for selecting the most suitable hybrids in areas where GLS is problematic.

Fungicide treatment reduced overall disease severity, with the most susceptible hybrids having the highest responses in the control of disease (leaf-blighting) and gain in yield (Table 3). Under the low disease levels in 1992/93, these responses were not as apparent as under high disease levels in 1993/94. The regression analysis indicated hybrids such as RS 5206 to be highly susceptible to GLS, since the level of disease was higher than predicted by the analysis. Gain in yield was however also higher (50%) than predicted. This high-yielding hybrid was previously widely grown by farmers, and had significantly reduced yields in the presence of GLS. SNK 2888, was considered to be tolerant of GLS, since the level of disease was higher than predicted, but with the surprisingly high unsprayed yield, the gain in yield was, as or lower than predicted by the model. In contrast, hybrids such as PAN 6480 and NS 9100 with lower than predicted disease, and which had predicted or lower than predicted gain in yield due to fungicide treatment indicating a lesser susceptibility to GLS.

GLS disease at Cedara usually infects maize at or near anthesis. The loss of photosynthetic leaf-area from leaf-blighting translates into loss in grain-yield. Fungicides delay the development of disease and treatment in areas prone to GLS produce yield gains (Ward *et al.*, 1997. This was true across seasons, although the yield responses to fungicides were lower in the dry season of

1992/93, which resulted in lower levels of disease. The ability to predict yield responses of hybrids to fungicide treatment under varying levels of disease can assist in deciding whether to apply fungicides. For instance, is fungicide treatment of maize warranted when disease-levels are relatively low in dry-seasons which are infavourable for GLS? The model predicted a response of 1641 kg ha^{-1} to fungicides when the mean disease level near physiological maturity was only 31%. With lower disease levels of 18%, the predicted yield response of PAN 6480 was 960 kg ha^{-1}. Being a less susceptible hybrid the actual response was lower, 416 kg ha^{-1}. This response was the result of three fungicide treatments, and, at current maize prices, the break-even yield for fungicide treatment is 290 kg ha^{-1} per treatment. In this instance, spraying would not be economical as the cost of three fungicide treatments would have exceeded the actual yield response. In contrast, the higher than average disease-level of 53% of RS 5206 in 1992/93 resulted in a predicted yield response of 2 837 kg ha^{-1}. With an actual response of 2 292 kg ha^{-1}, the economics of treatment is justified. However, under low-disease levels it would be more economical to produce grain from unsprayed PAN 6480 than RS 5206 with three spray treatments. The situation under high disease pressure is different. PAN 6480, with 75% leaf-blighting, the yield response predicted by the model was 3 855 kg ha^{-1}. Three fungicide applications, with a break-even yield response of 870 kg ha^{-1}, is therefore justified. There is, however, less risk in selecting hybrids less susceptible to GLS than more susceptible hybrids like RS 5206, which may have a higher yield response to fungicide sprays. Ward *et al.* (1997), showed that disease develops earlier and more rapidly in more susceptible hybrids. These are likely to require more spray treatments than less susceptible hybrids in which disease is later and slower in developing. The additional sprays require additional costs, and if the timing of the sprays is delayed there would be greater risk of yield losses. This might negate the higher yield potential of a susceptible hybrid such as RS 5206.

The timing of the initial fungicide spray relative to disease severity is to be important to the effectiveness of the treatment (Ward *et al.*, 1997). The initial spray was applied when disease severities, estimated by the logistic model (Vanderplank, 1963), varied between 0,5 and 1,5 percent (results not presented). These disease severity levels were within the threshold limits prescribed for initial spray treatment (Ward *et al.*, 1997), and the frequency and intervals between spray applications were sufficient to provide optimum control of GLS.

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CHAPTER 5

CHEMICAL CONTROL OF MAIZE GREY LEAF SPOT[(1)5]

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Grey leaf spot, *Cercospora zeae-maydis*, has, in a relatively short period, caused significant annual grain yield losses in the maize industry of KwaZulu-Natal, South Africa. No commercial hybrids are resistant to the disease, and because alternative measures of control, such as crop rotations and tillage practices, have limited effects, fungicides are the main option available for the control of the disease. Benomyl was initially registered for the control of grey leaf spot, but, because of possible development of fungicide resistant strains of *C. zeae-maydis* to the benzimidazole chemical group of fungicides, alternative fungicides, with different modes of action, were investigated. This study was initiated to establish which fungicides and fungicide mixtures, with different modes of action, would control grey leaf spot effectively, and delay the possible development of pathogen resistance. Fungicides belonging to the triazole chemical group, and combinations of fungicides of the benzimidazole and triazole group, were highly effective. The combination of the two fungicide groups, with their different modes of action, not only provide excellent control of GLS, but offer the benefit of slowing down development of pathogen resistance to fungicides. Lower than

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recommended rates of fungicides tested resulted in higher disease and lower grain yields. Recommended rates for difenoconazole and carbendazim were optimum for the control of GLS. However, recommended rates tested for benomyl, flutriafol and flusilazole/carbendazim were less than optimum for the control of GLS.

Keywords: Grey leaf spot disease; fungicides; maize; *Cercospora zeae-maydis*

Running Title: Chemical control of maize grey leaf spot

Introduction

There can be few diseases of major crops that have become major threats to economical crop production as rapidly as has grey leaf spot (GLS) of maize (*Zea mays* L.) (Smith, 1989). GLS is caused by the fungus *Cercospora zeae-maydis* Tehon and E Y Daniels. The disease, first observed in 1988 in KwaZulu-Natal, has since spread rapidly throughout the province and has been observed in neighbouring provinces and countries. In 1990/91, severe economic damage to crops was first reported (Ward and Nowell, 1997). The first fungicide trials, conducted at the Cedara Agricultural Development Institute (CADI), near Pietermaritzburg, South Africa, in the 1991/92 season, showed that the disease is capable of reducing grain yields by 20 to 60%, but systemic fungicides were found to provide excellent control (Ward, Birch and Nowell, 1994).

Maize is the only known host of *C. zeae-maydis* and the pathogen overwinters only in infected maize residues (Beckman and Payne, 1982; Latterell and Rossi, 1983). Not surprisingly, the increase in incidence and severity of GLS has been linked to maize grown under monoculture and conservation tillage practices that leave infected maize residues on the soil surface (Rupe, Siegel and Hartman, 1982; Stromberg and Donahue, 1986; Payne, Duncan and Adkins, 1987; Anderson, 1995). Disease levels increase with the amount of residue on the soil surface (de Nazareno, Lipps and Madden, 1993; Perkins, Smith, Kinsey and Dowden, 1995).

Tillage practices aimed at the complete burial of infected maize residues have been demonstrated as a means of controlling GLS (Payne and Waldron, 1983; Huff, Ayres and Hill, 1988). However, in recent seasons the disease has been observed to be a problem conventionally ploughed fields (Perkins *et al.*, 1995). In South Africa, no commercial hybrids are resistant to GLS and, because alternative methods such as tillage practices and crop rotations have limited control of GLS, chemical control measures offer an interim solution (Ward and Nowell, 1997). At the 1994/95 maize-grain price of R650 ton^{-1}, the break-even yield to cover costs of fungicide treatment of R135 ha^{-1} is 210 kg of grain ha^{-1} per treatment. This makes the use of fungicides economic in commercial maize production in South Africa. Benomyl fungicide was registered for the control of GLS following trials conducted at Cedara in 1990/91. However, problems with resistance in pathogen populations, especially to the benzimidazole group of fungicides, have become common in many crops, and in some cases resistance has developed rapidly (Smith, 1988). To preserve the effective lifespan of the fungicides in controlling GLS, resistance management strategies are advisable. The use of single component chemicals with site specific modes of action should be avoided. Mixtures of unrelated fungicides with different modes of action is the basic component of fungicide resistance management. Rotations of fungicides with different modes of action is an alternative strategy (Delp, 1988).

The present investigation was undertaken to establish which fungicides and mixtures of fungicides, with different modes of action, are most effective in the control of GLS. A rate of application trial was initiated to establish optimum rates for treatment.

Materials and methods

Two trials were conducted at CADI, Cedara (29° 31'S, 30° 17'E and alt. 1070 m). Evaluations of different fungicides and fungicide mixtures (FUNG. EVALUATION 92/93 and FUNG. EVALUATION 93/94) were conducted in 1992/93 and 1993/94, respectively. The experiment was repeated in 1994/95, but due to low disease levels induced by the prevailing drought, results were discarded because of heteroscedasticity of variance. A second trial studying the rates of application of fungicides (FUNG. RATES 92/93) was conducted in 1992/93. Maize had previously been grown on the site before the first trials were undertaken in 1992/93. The fields had abundant maize debris, naturally infested with *C. zeae-maydis* from the previous season. The trials were no-till planted to a GLS susceptible hybrid RS 5206 with a John Deere 7000 four-row, Max-Emerge planter, at a rate of 50 000 seeds ha^{-1}. Final plant-stands were 47 500 plants ha^{-1}. Fertilizer sufficient for an eight-ton grain crop was band-applied at planting. A top-dressing of 100 kg N ha^{-1} was broadcast when maize was 750 mm high. Normal pest- and weed-control practices for the area were followed. Plots comprised eight 9,0 m rows spaced 750 mm apart. The FUNG. EVALUATION 1992/93 and 1993/94 trial comprised randomised complete blocks designs, replicated three times in 1992/93 and four times in 1993/94. The FUNG. RATES 1992/93 trial comprised four replications in a randomised complete blocks design. The central four rows of each plot were sprayed with the fungicides, and the central two 8,0 m rows were hand-harvested. Grain yields were adjusted to a moisture content of 12,5% and expressed as kg ha^{-1}. Crude protein analysis was conducted for the grain yields in 1992/93.

The 1992/93 growing season was characterised by a lack of rainfall, and by hot days. The weather conditions until early grainfill were hot and dry and it was only in mid- to late-grainfill, after mid-February, that rainfall normalised. In contrast, rainfall during the 1993/94 season was above-average and well-distributed throughout the growing season. Mists were abundant, especially in January and February. Temperatures during the vegetative stages of growth were average, but were lower than the mean during grainfill (Table 1).

Table 1. Rainfall and temperature at Cedara for the 1992/93 and 1993/94 growing seasons

	Oct	Nov	Dec	Jan	Feb	Mar	Apr	Total
Rainfall (mm)								
1992/93	34	83	69	69	108	115	25	503
1993/94	133	63	162	206	127	113	37	846
Mean monthly*	125	116	126	158	130	103	32	789
Mean temperature								
1992/93	17.7	18.5	20.3	21.0	20.0	19.4	17.3	19.2
1993/94	17.0	18.3	19.4	19.9	19.4	19.0	17.1	18.6
Mean monthly*	17.1	18.4	19.9	20.6	20.4	19.2	17.6	19.0

* Long-term mean from 1914 to 1996

Planting dates and fungicide treatment

The FUNG. RATE 1992/93 trial was planted on 26 November 1992, and fungicide applications were made 64 and 83 days after planting (DAP). The FUNG. EVALUATION 92/93 trial was planted on 26 November 1992 and fungicides applied 76 and 104 DAP. The FUNG. EVALUATION trial 93/94 was planted on 17 November 1993, and fungicides applied 72 and 98 DAP.

Fungicide treatments were selected from commercially available products of the triazole and benzimidazole chemical groups (Table 2). These groups were chosen as they were known to be effective against GLS (Stromberg, 1990). Benomyl, belonging to the benzimidazole fungicide group, was registered for the control of GLS in 1991/92. It was chosen as the fungicide control against which the performance of other fungicides could be compared. The FUNG. EVALUATION 92/93 included difenoconazole, flutriafol, propiconazole and flusilazole, which were fungicides of the triazole group and rates suggested by manufacturers and were commercially available and likely to be effective for the control of *Cercospora*. Combination products, carbendazim/flutriafol and carbendazim/flusilazole, were suggested as likely to be more active than single component products. In 1993/94, propiconazole, flusilazole, and flusilazole/ carbendazim combinations were discarded for commercial reasons, whilst tebuconazole was discarded because of lack of efficacy. Carbendazim/difenoconazole was included in 1993/94 at the request of the manufacturers, whilst carbendazim alone was included because the combination products containing carbendazim had proved highly effective in controlling disease in the previous season. Copper sulphate pentahydrate was included at the request of the University of Natal in 1993/94. Spray solutions were applied with a CO_2-pressurised back-pack sprayer fitted with a vertically mounted spray-boom with three Whirlrain ¼" WRW2-20° nozzles spaced one metre apart. Full-cover sprays of 450 L ha^{-1} at 2 Bar pressure were applied to each maize row. Separate aerial spray trials (not reported) applying products at rates tested in spray volumes fo 40 L ha^{-1} have been effective in the control of GLS.

able 2. Fungicides evaluated at Cedara in the 1992/93 and 1993/94 seasons

Trade Name	Manufacturer	Active ingredient (ai)	Formulation[(a)] and ai content	Rate applied (g ai ha^{-1})	
				Rates trial[(b)]	Fungicide trial
Benlate	Du Pont de Nemours	benomyl	WP, 500 g ka^{-1}	125 (0.5X) 250 (1.0X) 500 (2.0X)	375
Capitan	Du Pont de Nemours	flusilazole	SC, 125 g L^{-1}		125
Early Impact	Zeneca	carbendazim/flutriafol	SC, 150/94 g l^{-1}		187/117.5
Eria	Ciba-Geigy	carbendazim/difenoconazole	SC, 125/62.5 g L^{-1}		125/62.5
Folicur	Bayer	tebuconazole	EC, 250 g L^{-1}		250
Impact	Zeneca	flutriafol	SC, 125 g L^{-1}	62.5 (0.5X) 125 (1.0X) 250 (2.0X)	156
Punch C	Du Pont de Nemours	flusilazole/carbendazim	SC, 125/250 g L^{-1}	62.5/125 (0.5X) 125/250 (1.0X) 250/500 (2.0X)	125/250
Punch Xtra	Du Pont de Nemours	carbendazim/flusilazole	SC, 250/125 g L^{-1}	125/62.5 (0.5X) 250/125 (1.0X) 500/250 (2.0X)	250/125
Score	Ciba-Geigy	difenoconazole	EC, 250 g L^{-1}	37.5 (0.5X) 76 (1.0X) 150 (2.0X)	87.5
Tilt	Ciba-Geigy	propiconazole	EC, 250 g L^{-1}		250
		copper sulphate pentahydrate	S, 85.4 g L^{-1}		85.4

WP, wettable powder; SC, suspension concentrate; EC, emulsifiable concentrate; S, solution.
0.5X, half-recommended; 1.0X, recommended; 2.0X, double recommended rate.

Disease assessment

Whole plant standard area diagrams described by Ward, Laing and Rijkenberg (1997) were used as a guide for estimating disease percentage severity. Disease severity assessments were made regularly at 10 to 14 day intervals on plants in the centre of the two middle rows of each plot, from the first signs of disease, and continued until the crop was physiologically mature. These data were used in calculating the area under disease progress curve (AUDPC), which is the summary of the disease epidemic. The AUDPC was calculated using a trapezoidal integration program (Berger, 1981) and was standardised (SAUDPC) by dividing the AUDPC value by the duration of the epidemic. The SAUDPC allows for comparisons of disease from one season to the next. Percent disease severity data were transformed to fit the logistic model (Vanderplank, 1963). The model described the disease progress, estimated infection rates (r), and was used to estimate the duration of fungicide control (Ward *et al.*, 1997).

Statistical analysis

Statistical analysis of trial data was conducted by analysis of variance (ANOVA) and mean separations were based on least significant difference (LSD) at the 5% level of probability and orthogonal contrasts at the 5% level of probability were conducted on the results of the FUNG. EVALUATION trial.

Results

Disease was first observed in the trials 69 DAP in 1992/93, and 59 DAP in 1993/94. Physiological maturity occurred 145 DAP.

Fungicide evaluation, 1992/93 season

Fungicide treatments reduced infection rate, disease (SAUDPC) and final disease severity. Amongst the most effective treatments was carbendazim/flusilazole, whilst tebuconazole was least effective (Table 3). Carbendazim/flusilazole (30.8 days) provided amongst the longest duration of control of the products carbendazim/flutriafol, flusilazole/carbendazim, flusilazole, propiconazole and benomyl. These products provided longer control than tebuconazole (3.0 days) and propiconazole (20.0 days) (Table 3). All fungicide treatments resulted in higher grain yields than the unsprayed treatment. Only the tebuconazole treated plots had lower grain yields than those treated with other fungicides, except flusilazole/carbendazim.

Surprisingly, the combination products with carbendazim, and the benzimidazoles (carbendazim and benomyl) resulted in lower grain crude protein than the triazole single fungicide products and the unsprayed treatments (Table 3). The reasons for this finding is at present not known.

Table 3. Grey leaf spot disease severity, infection rate, SAUCPC values, effective period of control and grain yield (kg ha^{-1}) for various fungicides during the 1992/93 and 1993/94 seasons

Treatment	Grey Leaf Spot								Grain yield		
	Infection rate (r x 100)		Disease SAUDPC[a]		Final disease severity [b]		Effective period of control[c] (days)		% Crude Protein	Grain yield (tons ha^{-1})	
	92/93	93/94	92/93	93/94	92/93	93/94	92/93	93/94	92/93	92/93	93/94
Untreated control	16.52 a[d]	10.90 a	41.8 a	43.9 a	83.7 a	90.0 a	0.0 e	0.0 e	9.995 a	4.227 a	4.858 a
Benomyl[e]	6.90 b	5.87 b	8.3 de	7.8 d	18.1 cde	28.1 c	21.0 ab	18.0 bc	9.313 c	7.319 c	6.964 b
Difenoconazole	7.37 bc	7.45 b	15.8 c	15.7 c	32.5 c	53.8 b	10.0 cde	14.0 cd	9.775 ab	7.731 c	7.205 b
Flutriafol	6.87 bc	6.57 b	8.1 de	10.4 cd	12.5 de	34.7 c	13.5 bcd	18.5 bc	9.620 abc	7.723 c	6.995 b
Carbendazim/flutriafol	6.35 bc	7.25 b	6.5 de	7.6 d	8.6 de	33.1 c	28.3 a	21.0 abc	9.485 bc	7.568 c	7.810 b
Carbendazim/flusilazole	4.30 c	6.07 b	4.7 e	8.3 d	4.2 e	26.2 c	30.8 a	26.0 ab	9.502 bc	7.727 c	7.411 b
Flusilazole/carbendazim	5.57 bc		6.9 de		8.9 de		22.8 a		9.575 bc	6.882 bc	
Propiconazole	7.20 bc		11.1 cd		23.1 cd		20.5 abc		9.743 ab	7.580 c	
Tebuconazole	9.70 b		25.3 b		53.7 b		3.0 cde		9.788 ab	5.913 b	
Flusilazole	6.37 bc		8.6 de		11.9 de		25.3 a		9.438 bc	7.948 c	
Copper Sulphate pentahydrate		11.50 a		33.4 b		87.5 a		5.8 de			4.482 a
Carbendazim/difenoconazole		6.32 b		8.6 cd		26.6 c		25.3 ab			7.561 b
Carbendazim		6.65 b		6.1 d		28.8 c		28.0 a			7.456 b
Mean	7.72	7.58	13.7	15.2	25.6	44.2	17.5	17.3	9.623	7.062	6.874
L S D (0.05)	4.61	1.76	5.1	7.1	17.1	4.61	12.9	10.7	0.411	1.274	1.348
% CV	29.1	11.3	17.9	22.6	32.4	29.1	35.8	30.1	2.1	8.8	9.7

[a] SAUDPC - area under disease progress curve, standardised (SAUDP) by dividing AUDPC value by duration of epidemic

[b] Final disease severity is percent leaf-blighting near physiological maturity

[c] Duration of fungicide control, calculated from the logistic model

[d] Values followed by the same letter in the same column are not significantly different (P = 0.05)

[e] Treatments applied at current registered or manufacturer's suggested rates.

Fungicide evaluation, 1993/94 season

All fungicides, except copper sulphate pentahydrate, reduced infection rate, disease (SAUDPC) and final disease severity. Difenoconazole and copper sulphate pentahydrate had higher disease severity than other fungicide treatments. Carbendazim and the combination products had amongst the longest duration of control of the fungicides evaluated, whilst, difenoconazole and the copper treatment had amongst the shortest duration of control (Table 3).

Fungicide rates evaluation, 1992/93 season

Effect of fungicides. Fungicides reduced the mean infection rate and disease (AUDPC), and this was reflected in lower final severity and higher grain yields than the unsprayed control (Table 4). There were no differences in the means of infection rates and disease between the fungicides evaluated. Flutriafol had amongst the lowest disease (5.8%), whilst difenoconazole (14.4%) had amongst the highest disease severity of the fungicides (Table 5). Carbendazim/flusilazole provided, amongst other treatments, the longest duration of control (66.1 days). In spite of relatively high final disease, difenoconazole had amongst the highest grain yields of the fungicides evaluated (Tables 4 and 5).

Table 4. The effective period of control and grain yield for different rates of application of fungicides during the 1992/93 season

Treatment	Effective Period of Control (days)				Grain Yield (tons ha^{-1})			
	0.5X(a)	1.0X	2.0X	Mean	0.5X	1.0X	2.0X	Mean
Untreated control	0.00	0.00	0.00	0.00 a(b)	5.899	5.659	6.159	5.903 a
Benomyl	55.67	58.67	66.00	60.11 b	7.022	8.229	8.033	7.762 b
Difenoconazole	64.67	63.67	63.00	63.78 bc	8.096	9.137	9.024	8.752 c
Flutriafol	58.33	62.67	69.00	63.33 bc	7.521	9.309	8.610	8.480 bc
Carbendazim/flusilazole	63.33	68.33	66.67	66.11 c	8.156	8.672	8.684	8.504 bc
Flusilazole/carbendazim	52.33	52.33	69.67	61.44 bc	7.575	8.424	9.059	8.353 bc
Mean	49.06 a	52.61 ab	55.72 b	52.46	7.347 a	8.238 b	8.261 b	7.959
$LSD_{0.05}$ between fungicides	5.55				0.771			
$LSD_{0.05}$ between rates	3.64				0.506			
$LSD_{0.05}$ within rates and fungicides	10.83				1.504			
% C V	9.9				9.1			

Notes:
(a)Rates at which fungicides were applied:
0.5X=half the recommended rate
1.0X=the recommended rate
2.0X=double the recommended rate

(b)Values followed by the same letter in columns or rows are not significantly different (<0.05)

Table 5. **The infection rate (r values), AUDPC values, and final disease severity for different rates of application of fungicides during the 1992/93 season**

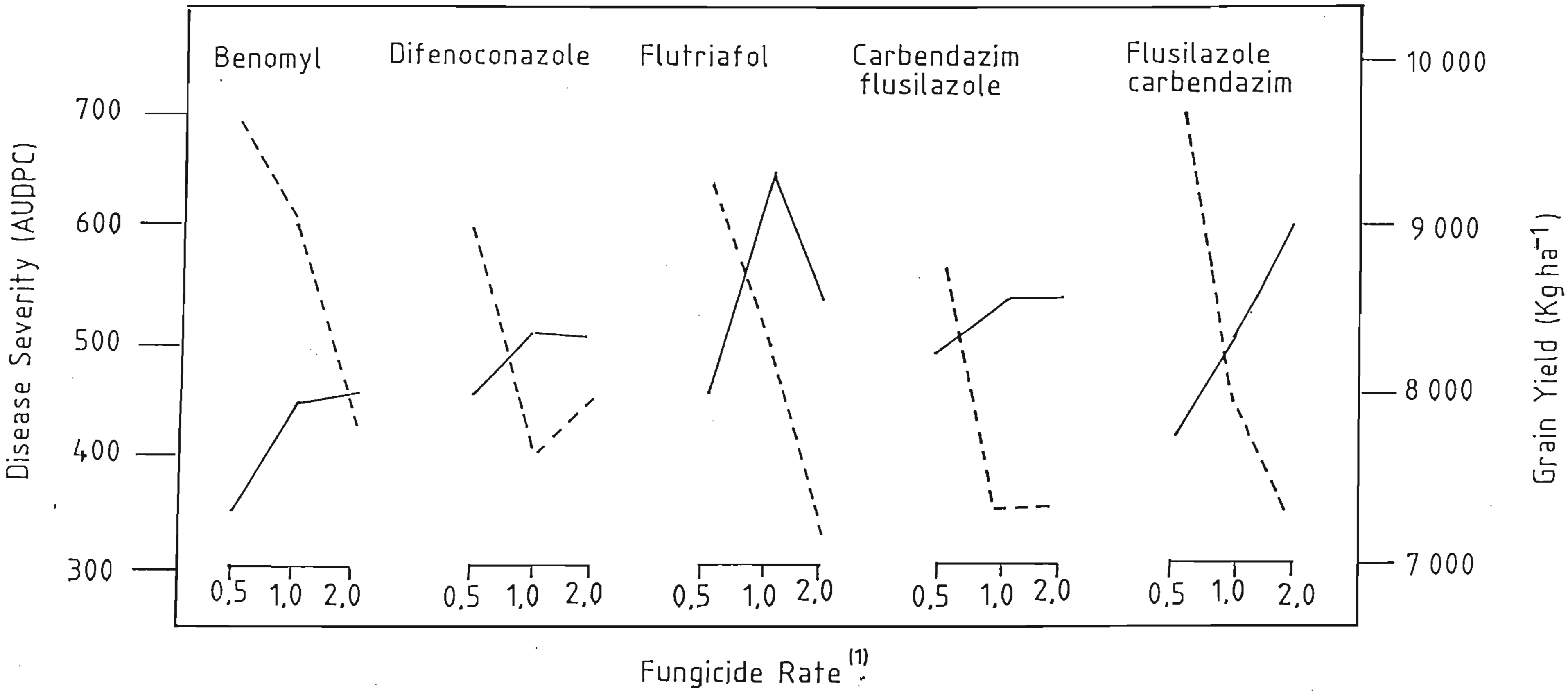
Treatment	Infection Rate (r x 100)(a)				Disease (AUDPC)(b)				Final disease severity (%)(c)			
	0.5(d)	1.0X	2.0X	Mean	0.5X	1.0X	2.0X	Mean	0.5X	1.0X	2.0X	Mean
Untreated control	9.07	9.07	8.73	8.96 a(e)	2113	2193	2061	2122 a	75.2	75.0	66.7	72.3 a
Benomyl	5.67	5.63	4.30	5.20 b	692	602	432	575 b	20.0	9.3	2.3	10.6 bc
Difenoconazole	5.67	3.77	4.80	4.74 b	619	408	450	493 b	20.8	15.0	7.3	14.4 b
Flutriafol	5.93	5.27	3.53	4.91 b	642	512	335	496 b	9.3	3.3	4.8	5.8 c
Carbendazim/flusilazole	5.57	4.13	4.13	4.61 b	567	357	359	428 b	16.5	4.7	8.3	9.8 bc
Flusilazole/carbendazim	6.37	4.80	3.57	4.91 b	761	458	345	521 b	12.5	9.0	3.3	8.3 bc
Mean	6.38 a	5.44 b	4.84 b	5.56	899 a	755 b	664 b	773	25.7 a	19.4 b	15.5 b	20.2
$\text{LSD}_{0.05}$ between fungicides	1.05				197				7.9			
$\text{LSD}_{0.05}$ between rates	0.69				129				5.2			
$\text{LSD}_{0.05}$ within rates and fungicides	2.05				384				15.0			
% C V	17.7				23.7				36.6			

Notes:
(a)r value is = linear rate of increase in GLS x 100
(b)AUDPC is the area under disease progress curve
(c)Final disease severity is percent leaf-blighting near physiological maturity
(d)Rates at which fungicides were applied:
0.5X = half the recommended rate
1.0X = the recommended rate
2.0X = double the recommended rate
(e)Values followed by the same letter in columns or lows are not significantly different (P<0.05)

Responses to rates of application. The mean of the half-recommended rate (0.5X) had higher infection rates, more disease and higher final disease than recommended (1.0X) and double recommended (2.0X) rate. The 2.0X rate provided longer effective control (55.7 days) than 0.5X rate (49.1 days), but there was no difference in the length of control between 1.0X and 2.0X rates. Grain yields were lower at 0.5X than 1.0X and 2.0X rates (Tables 4 and 5).

There was a significant interaction between fungicides and rates of application. The responses of individual fungicides to rates applied (Tables 4 and 5) are illustrated graphically (Figure 1). When the disease continues to decline between 1.0X and 2.0X rates, the optimum rate is not indicated. Optimum rates are indicated when disease curve flattens between these rates. Similarly, optimum yields are not achieved when the yield response continues to climb with increasing rates. This is well illustrated with flusilazole/carbendazim (Figure 1). Disease continued to decline between 1.0X and 2.0X rates and yield continued to climb with increasing rates, indicating that optimum rates had not been achieved.

Benomyl reduced the infection rate, disease, disease severity and had longer effective control at 2.0X than 0.5X and 1.0X rates (Tables 4 and 5). This is shown in Figure 1, when disease continued to decline between 1.0X and 2.0X rates, but the grain yield response appeared optimal. Overall, the results indicated that 1.0X rate was less than optimum.



(1) 0,5 = half recommended rate 1,0 = recommended rate 2,0 = Double recommended rate

Figure 1 The effect of rate of application of different fungicides on disease severity (- - -) and Grain yield (kg ha^{-1}) (——)

Difenoconazole resulted, surprisingly, a lower infection rate at 1.0X than 0.5X and 2.0X rates. There were no differences in disease and length of control between 1.0X and 2.0X rates. The 2.0X rate, however, resulted in lower final disease than 1.0X rate, but this was not reflected in yield (Tables 4 and 5, Figure 1). Optimum rates appeared to be the recommended 1.0X rate.

Flutriafol resulted in a lower infection rate, lower disease and longer effective control at 2.0X than 1.0X rates. There was, surprisingly, lower final disease severity and higher grain yield at 1.0X than 2.0X rates (Tables 4 and 5, Figure 1). Overall the results indicated 1.0X rate to be lower than optimum.

The combination, carbendazim/flusilazole, had no infection rate responses, nor were there disease, disease severity, length of control and grain yield responses to increased rates above recommended (1.0X) rate. This indicated that recommended rates were optimum. The flusilazole/carbendazim combination, on the other hand, responded to increased rates, indicating that 1.0X rate was not optimum.

Discussion

Fungicides were highly effective in both seasons in controlling GLS and increasing grain yields. The exception to this was copper sulphate pentahydrate, which was less effective in controlling disease, with concomitant lower grain yields, than other fungicide treatments in 1993/94. It is unfortunate that results of a third season's experiments in 1994/95 were abandoned due to severe drought. However, results from fungicide treatments that were common over the two seasons' experiments were sufficiently consistent to provide credence to the results.

All fungicides tested in the fungicide evaluation trial, except tebuconazole, difenoconazole and copper sulphate pentahydrate, provided as effective control of disease and final disease severity as the registered fungicide control, benomyl. These higher levels of disease and disease severity in the difenoconazole and tebuconazole treatments were not however, reflected in lower grain yields than benomyl. Copper sulphate pentahydrate treatments yields were lower than the fungicide control indicating the product to be less effective in controlling GLS. The combination products provided as effective control of GLS over the two seasons, and had as high grain yields (in 1993/94) than effective single component products. The combination products also provided longer effective control. Because of resistant management strategies in delaying pathogen resistance to fungicides, the combination products are preferred to single component products.

AUDPC and final disease severity are better parameters for evaluating fungicide performance than infection rate. These parameters related well to the effective length of control provided by fungicides, but did not always relate directly to the grain yields obtained. This is well illustrated with the fungicide difenoconazole, which had relatively more leaf-blighting (disease) and disease severity than other fungicides evaluated. Yet the difenoconazole treatments produced amongst the highest grain yields. This anomaly may be due to growth-promoting properties of the triazole fungicides, which act by sterol-inhibition and have growth-regulatory properties (Lonsdale and Kotze, 1993). In contrast, the benzimidazole's (carbendazim and benomyl) have been shown to have no effect on maize growth when used alone (Smith, 1989). The growth-regulating properties of the triazoles may account for the relatively higher grain yield of difenoconazole in spite of relatively higher leaf-blighting. A more likely explanation, however, may be broader spectrum control of diseases by the triazoles. Rust (*Puccinia sorghi* Schw.) was the only other foliar disease observed in the experiments in untreated maize. The level of disease, however, was low (less than 2.5% infection) and was not expected to influence yield. Other diseases, not observed, but which might have been controlled included root- and stalk- rotting fungi.

It is of interest that the benzimidazole fungicides and combination products containing carbendazim yielded lower crude protein than the triazole fungicides. It is not known whether the higher protein obtained with single component triazoles is related to their growth-regulatory properties. Further research on this aspect may add another parameter for selection of fungicides to control maize diseases.

The fungicide rate of application experiment was useful in determining the optimum rates of fungicide-application. Prior to the commencement of this study, no work had been undertaken on optimum rates of application of fungicides for GLS control. Benomyl, for example, was initially registered at 0.5 kg ha^{-1} active ingredient (ai), as this was the only rate at which the product had been tested previously. The results of the experiments in this study indicated responses to rates between 0.250 and 0.500 ai kg ha^{-1}. The product was subsequently registered for use at 0.375 kg ha^{-1}. The results obtained with the combination products indicated carbendazim/flusilazole to be more active against GLS at equivalent rates of active ingredient than flusilazole/carbendazim. For commercial reasons, the more active carbendazim/flusilazole combination was registered for use in preference to flusilazole/carbendazim.

Conclusions

All fungicides tested in this study, except copper sulphate pentahydrate, provided effective and economic control of GLS. Fungicide combinations of the benzimidazole and triazole groups, however, provided more effective and longer duration control than single component fungicides. For these reasons, and because of the history of pathogen resistance to the benzimidazole group of fungicides in other crops, the use of fungicide combinations is recommended as a resistance-management strategy. This is important for continued economic production of maize, since hybrids resistant to GLS have not yet been released in South Africa. Until such hybrids become available, farmers in areas where GLS is a problem will rely on fungicides if they are to continue to produce maize crops economically.

Since the commencement of these studies, and with supporting data from this trial, carbendazim/flusilazole, carbendazim/flutriafol, carbendazim/difenoconazole, flutriafol and difenoconazole have been registered for use on maize for the control of GLS. As a resistance-management strategy, benomyl and carbendazim are no longer registered for use on maize.

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CHAPTER 6

FREQUENCY AND TIMING OF FUNGICIDE APPLICATIONS FOR THE CONTROL OF GRAY LEAF SPOT IN MAIZE(1)6

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ABSTRACT

Ward, Laing, M.D. and J.M.J., Rijkenberg, 1995. The frequency and timing of fungicide applications for the control of gray leaf spot in maize. Plant Dis.:-

Time of application and frequency of fungicide treatments for management and control of *Cercospora zeae-maydis* were quantified using the logistic model and area under disease progress curve (AUDPC). Control was most effective when spraying commenced as disease severity levels reached 1 to 2% of the leaf area blighted, and when lesions were restricted on the basal five leaves of the maize plant. Highest grain yields were achieved with treatments providing disease control until the crop was physiologically mature. To provide this length of

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control, the frequency and number of fungicide applications varied with the stage of host development when disease was first apparent; with early infections, more fungicide treatments were necessary to provide protection until physiological maturity. Yield responses to fungicides appeared to be a function of the growth stage of the host when sprays are initiated, the amount of disease at spray date, the length of fungicide control and effective control through to physiological maturity.

INTRODUCTION

Few diseases of major crops have risen from relative obscurity to general recognition as a threat to economical production as suddenly as has gray leaf spot (GLS) of maize (*Zea-mays* L.). GLS is caused by the fungus *Cercospora zeae-maydis* Tehon and E Y Daniels (10). The disease was first observed in 1988 in KwaZulu-Natal, South Africa, and has since spread rapidly throughout the higher rainfall and irrigated areas of the province. In these areas, it is estimated that 60% of the commercial crop grown is affected by GLS (13). The pathogen has more recently been observed in major maize-growing areas of neighboring provinces in areas of lower rainfall. This is of great concern to the maize industry. Trials conducted at Cedara Agricultural Development Institute (CADI) near Pietermaritzburg, South Africa, have shown that the disease is capable of reducing grain yields by 20 to 60% depending on hybrid susceptibility to *C. zeae-maydis (14).* Fungicides can provide excellent control of the pathogen (14).

Disease progress for GLS is, in general, best described by the logistic model. Progress curves linking the transformed disease severity data points enables the examination of the effects of fungicide treatment and comparisons of disease progress of the different treatments. In general, application of an effective fungicide to foliage interrupts progress of a disease soon after spraying and lasts for an "effective period". This fungicide effective period (FEP) is defined as that period, after application of the fungicide, during which there is minimal disease increase and ends when there is a rapid increase in disease (3). The FEP is measured from disease progress curves of the transformed data of each treatment and starts from the time of fungicide application and ends when there is a sharp increase in disease progress and was determined graphically. This differs slightly from the description by Berger (3), who suggested that the treatment effect of a protectant fungicide starts one latent period after spraying, because latent infections are not controlled by protectant fungicides. The discrepancy arises because systemic fungicides used in this study control all infections, including latent infections. Characteristics of the theoretical response to fungicide treatment are the sharpness of the swing of the disease into the FEP, zero disease increase during the effective period and the parallelism of the disease progress to the non-treated control (the epidemic delay is equal to the FEP at all subsequent times after the FEP). The FEP is a function of fungicide efficiency, its dose, and the residual breakdown curve (3). With systemic fungicides the FEP is longer and the disease increase during the FEP is less than with protectant fungicides (3). In practice, when host growth is minimal, which is usual with GLS, as it attacks maize near anthesis, the FEP has a slight upward slant of increasing disease because of inability of the fungicide to provide complete control of the pathogen (3). The level of disease severity at the time of fungicide treatment affects subsequent disease progress. If disease severity is high at the time of application, the rate of disease increase into FEP is more gradual, the FEP has a sleeper slope (greater disease increase) and the FEP is shorter (3). At higher levels of disease, the infection efficiency becomes lower because there is less healthy tissue available for infection, although more inoculum may be present.

This investigation was undertaken to establish the most effective time of application of fungicides and the frequency of sprays necessary for effective control of GLS until physiological maturity.

MATERIALS AND METHODS

Field study. The study was conducted between 1991 and 1994 at CADI, (29° 32'S, 30° 17'E) situated 15 km north of Pietermaritzburg at an altitude of 1 070 m. The site was north-facing, gently sloping, and consisted of well drained deep sandy-clay loam soils of the Hutton form and Doveton series (8). The trial area had been continuously cropped to maize for the previous 10 years.

The trial was conducted on the same field over the three seasons. It was no-till seeded into the previous season's maize debris at a seeding rate of 50000 seeds ha^{-1} with a John Deere 7000 Max-Emerg 4-row planter. Four experiments were planted: on 31 October and 6 December in 1991; 5 November in 1992; and 26 October in 1993. Final plant stands were 45000 plants ha^{-1}. Fertilizer was applied at a rate sufficient for an 8-ton/ha^{-1} grain crop based on soil analyses and recommendations by the Cedara Fertilizer Advisory Service. Fertilizer was band-applied at planting to supply 32 kg N, 48 kg P, 63 kg K and 2.4 kg Zn ha^{-1}. Limestone ammonium nitrate was broadcast when maize was knee-high to provide a further 97 kg N ha^{-1}.

A tank-mix of metolachlor (1.86 g ai ha^{-1}) plus atrazine/metolachlor/ terbuthylazine (550/633/550 g ai ha^{-1}) was applied as a pre-emergent, overall treatment in 300 L water for the control of grasses and broadleaf weeds. Fenvalerate (28 g ai ha^{-1}) was included in the herbicide tank-mix for the control of cutworm. Carbofuran granules (2.7 kg ai ha^{-1}) were applied in the planting furrow for the control of soil insect pests.

Two experiments conducted in the 1991/92 season were split-plot design with four replications. Time of fungicide application was the main plot with hybrids as subplot treatments. Fungicides were applied as single spray applications made prior to, at, and after anthesis, 53, 67, 89 and 103 days after planting (DAP) in the early planted experiment, and 53, 67, 78 and 90 DAP in the late planting. Two hybrids were planted, National Seeds RS 5206 and Carnia's CRN 4526. RS 5206, previously widely grown in KwaZulu-Natal, is considered to be highly susceptible to GLS, whilst CRN 4526 is less susceptible. Following results from the 1991/92 experiments, the trial design in the 1992/93 and 1993/94 seasons was changed to a randomized complete blocks design. Treatments comprised single and multiple spray applications. The timing of the sprays commenced at different levels of disease, and were initiated when lesions were present on the basal two leaves, on the basal five leaves, on all basal leaves to ear-height and, on all basal leaves to above ear-height.

Plots comprised eight rows, spaced 750 mm apart and were 12 m long. Fungicide spray solutions were applied to the four central rows, and the central

10m of the two middle rows of the treated area were assessed for disease and harvested for grain yield. The fungicide used in 1991/92 was benomyl at a rate of 375 g ai ha^{-1}(Benlate 50% WP, Du Pont de Nemoirs and Coy). In the first season, it was applied in 1500 L ha^{-1} water at 0.15 kPa pressure with a hand-operated Cooper-Peglar back pack sprayer fitted with a lance and single Spraying Systems TeeJet No 8001 nozzle. In the subsequent seasons a combination of 187.50 g carbendazim plus 93,75 g flusilazole ai ha^{-1} was applied (Punch Xtra, Du Pont de Nemoirs and Coy) in 500 L water, using a CO_2-pressurized back pack sprayer with a vertically mounted spray-boom having three Whirlrain ¼" WRW2 - 20° nozzles spaced 1 m apart.

The dehusked ears from the central two rows of the trial unit were weighed in the field. Sub-samples of five or six ears were weighed and shelled in the laboratory, and the shelling percentage determined to calculate the shelled grain mass for each. Moisture content of a 250g sample of shelled grain was determined and the grain yield was adjusted to 12,5% moisture content.

Disease assessment. Kranz (7) and Campbell & Madden (4) stressed the importance of standard assessment diagrams as training tools and as guides to improve accuracy and precision in disease assessment. Standard diagrams were developed, based on the standard area diagrams by Smith (10). Leaves of a commonly grown maize hybrid at silking were numbered 1 to 17 for each leaf position from the bottom to the top of the plant. Surface area of each leaf for each leaf position of 20 plants was measured using a LI-COR LI-3100 Area Meter and surface area of an average leaf area for each position was established. Plants in the field showing GLS were examined, starting when only leaves 1 and

2 exhibited visual lesions. These were matched against Smith's standard area diagrams and the percent diseased tissue for each leaf was determined. Calculations using these data and the average surface area for the corresponding leaf position were used to establish the area of diseased tissue, as a percent of the whole plant. The procedure was repeated as the disease developed on leaves 3, 4 and 5 and progressively more leaves acropetally, until the entire plant exhibited gray leaf spot symptoms. Standard assessment diagrams showing 2, 5, 10, 20, 35 and 50% disease were constructed and used as a guide in estimating disease severity (Fig. 1). Disease severity assessments were made at 10 to 14 day intervals, commencing at first signs of disease until crop physiological maturity. These data were used to calculate the area under disease progress curve (AUDPC).

The AUDPC, a summary of the disease epidemic, was used for treatment comparisons and was calculated from data based on the trapezoidal integration program (2). For making comparisons between epidemics of different time durations, the AUDPC was standardized by dividing the AUDPC value by the total time (days) duration of the epidemic (6).

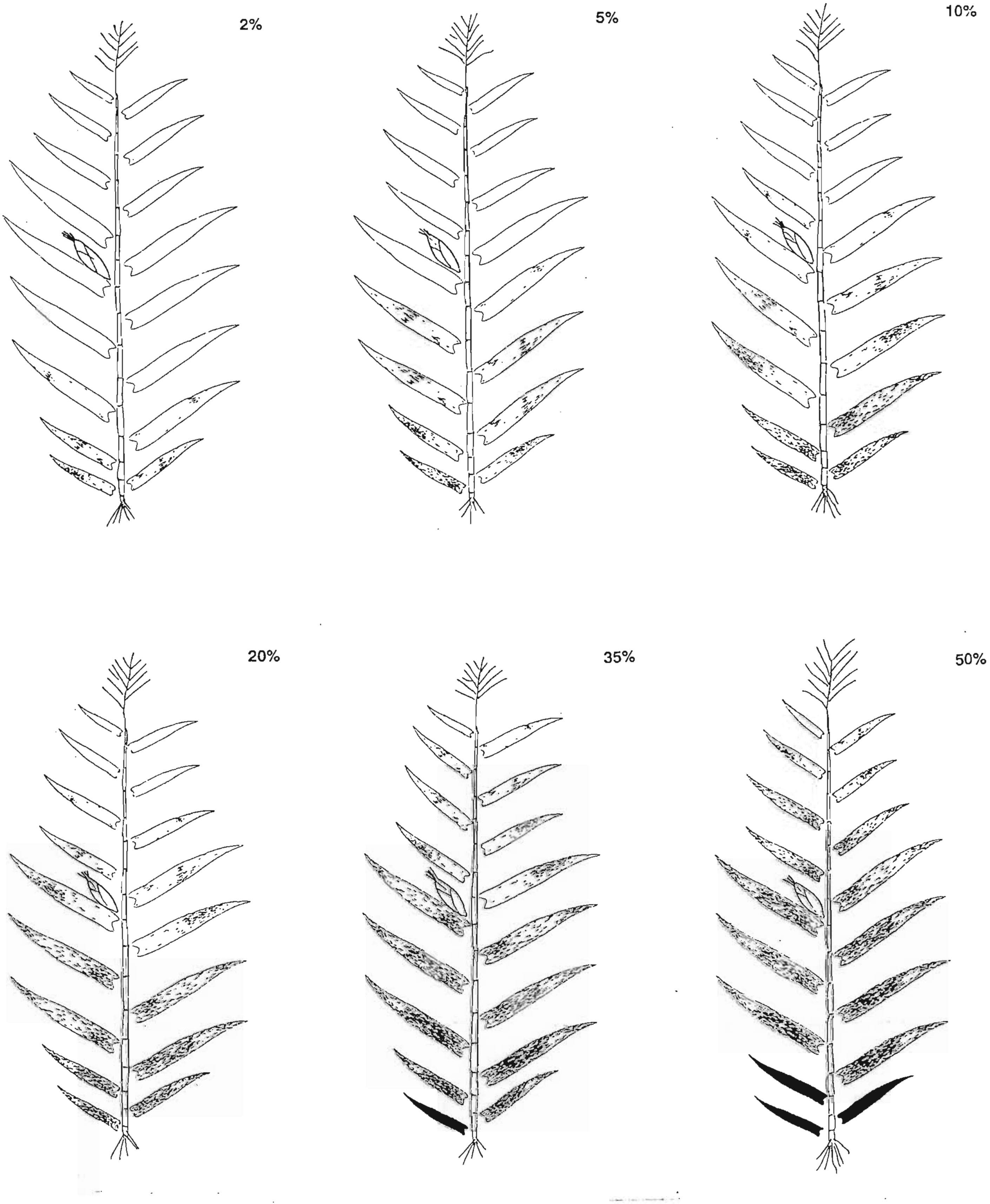

Figure 1. Standard assessment diagrams for evaluation of gray leaf spot lesion area on maize. (Percentages given represent the amount of leaf area affected and are indicated by the darkened portion of the leaf).

Statistical analysis. The disease severity data was transformed to fit the logistic model described by Vanderplank (12) and the Gompertz model described by Berger (2) in order to linearize the progress-curve, thereby allowing treatment comparisons, and the measurements of infection rate and FEP. Disease progress, except for the 1991/92 season, was best described by the Vanderplank model (based on comparisons of coefficients of determination (R^2)), and was used in this study. Disease severity, AUDPC, infection rate (r), FEP and grain yield were analyzed by analysis of variance (ANOVA). Mean separations were based on Duncan's multiple range test at the 5% level of probability. The analysis was conducted on Genstat 2.2. (Rothamstead Experiment Station, Harpendon, U.K.).

RESULTS

GLS was not observed at CADI prior to the 1991/92 season and inoculum levels *in situ* were assumed to be relatively low. However, the inoculum buildup, from the debris from this crop was sufficient to initiate GLS epidemics in subsequent seasons. GLS was first observed on 4 February, 4 January and 16 December in the 1991/92, the 1992/93 and the 1993/94 seasons, respectively. Anthesis was observed 89 and 77 DAP in the 1991/92 experiments and 80 and 78 DAP in the 1992/93 and 1993/94 experiments, respectively. Physiological maturity in these experiments occurred 150, 150, 149 and 153 DAP, respectively.

Weather conditions varied over the seasons in which the trial was conducted (Table 1). Growing conditions in 1991/92 were warm and moist during the vegetative growth stages of maize, but after anthesis, rainfall declined. During grainfill, days were warm to hot and heavy dews favoring disease were frequent.

The 1992/93 season was dry, with only 63% of the average rainfall recorded during the growing season. In contrast, the rainfall in 1993/94 was above average and well distributed throughout the growing season. Temperatures were lower than average during grainfill and mists were frequent, especially during January and February 1994.

Table 1. Rainfall and temperature at Cedara for the maize growing seasons 1991 to 1994.

	Oct	Nov	Dec	Jan	Feb	Mar	Apr	Total
Rainfall (mm)								
1991/92	209	198	149	105	120	95	51	927
1992/93	34	83	69	69	108	115	25	503
1993/94	133	68	162	206	127	113	37	846
Mean (50 year)	125	116	126	158	130	102	32	789
Mean Temperature (°C)								
1991/92	16.1	18.3	19.7	20.4	21.0	19.8	18.9	
1992/93	17.7	18.5	20.3	21.0	20.0	19.4	17.3	
1993/94	17.0	18.3	19.4	19.9	19.4	19.0	17.1	
Mean (50 year)	17.1	18.4	19.9	20.6	20.4	19.2	17.6	

1991/92 Season. Disease was first observed in the early and late planted experiments 95 DAP and 60 DAP, respectively. The fungicide treatments applied soon after disease was first observed in both plantings, on 11 February, provided amongst the most effective control of disease (AUDPC) and amongst the highest grain yields (Tables 2 and 3). As a result of more disease observations in the late planted experiment (6 December, 1991) presentation of results is confined to this experiment (Table 3).

Initial disease progress was slow, with disease severity of non-sprayed treatments increasing in the first 26 days from 1% to 8% in RS 5206 and from 1% to 3% in CRN 4526. Subsequent progress was rapid: disease severity increased in the following 30 days from 8% to 88% in RS 5206 and from 3% to 80% in CRN 4526 (Table 3).

Although there was on average higher disease (AUDPC) in RS 5206 than CRN 4526, RS 5206 had on average higher grain yields (6480 kg ha^{-1}) than CRN 4526 (5384 kg ha^{-1}) (Table 3). There was lower disease (AUDPC) in fungicide treatments of both hybrids than in the non-sprayed treatments, and this was reflected in highly significant grain yield responses to fungicides. The fungicide treatment initiated on 11 February, seven days after disease was first observed, had the lowest disease (AUDPC = 19) and the longest FEP (39 days) in RS 5206 and, had amongst the lowest disease (AUDPC = 16) and longest FEP (32 days) in CRN 4526. This treatment, 67 DAP, also had the highest grain yields in both hybrids, although these were not significantly different from fungicide treatments applied later.

The fungicide treatment applied, at 53 DAP, 2 weeks before disease was observed, had higher final disease severity, higher AUDPC, higher infection rate, shorter FEP, and lower grain yield than fungicide treatment at 67 DAP. Treatment later, at 90 DAP, when there was relatively higher final disease severity, also had higher AUDPC, shorter FEP and with CRN 4526 had lower grain yield than treatment at 67 DAP (Table 3).

Table 2. Gray leaf spot (GLS) disease development and grain yield of two maize hybrids (planted 31 Oct 1991) for fungicide treatments applied at different dates after planting (DAP[a])

Fungicide application details		Disease severity (%)[b]				Grain yield
Hybrid and date applied	DAP	Assessment (DAP)		r[c]	S AUDPC[d]	Kg ha^{-1}
		121	134			
RS 5206[e]						
Non-sprayed	-	22 a[f]	91 a[f]	28.4 a[f]	56 a [f]	6177 b [f]
23 Dec	53	24 a	86 a	23.5 a	55 a	5898 b
6 Jan	67	4 b	55 b	27.8 a	30 b	8480 a
28 Jan	89	1 b	16 c	24.0 a	9 c	9689 a
11 Feb	103	2 b	5 d	6.3 b	4 c	8686 a
Mean						7786 [g]
CRN 4526[e]						
Non-sprayed	-	16 c	91 a	31.6 c	54 e	3557 c
23 Dec	53	16 c	85 a	27.6 c	51 e	3875 bc
6 Jan	67	3 d	50 b	27.3 c	27 f	4575 b
28 Jan	89	1 d	13 c	21.2 d	7 g	4838 a
11 Feb	103	4 d	2 d	4.1 e	3 g	5360 a
Mean						4441 [g]
SE (of a mean)		1.5	3.3	3.0	1.8	363.8
Grand mean		9.5	49.6	21.4	29.5	6113
CV %		22.0	9.0	19.2	10.5	11.6

(a) Days after planting
(b) Disease severity is percent leaf tissue with symptoms of GLS
(c) r = infection rate x100, calculated by regressing the logistic transformation on time (DAP)
(d) Standardised area under disease progress curve
(e) Anthesis occurred 89 days after planting
(f) Means within a column having letters in common do not differ significantly ($P < 0.05$). Duncan's multiple range test
(g) The grain yield of RS 5206 was significantly greater ($P < 0.001$) than CRN 4526

Table 3. Gray leaf spot (GLS) disease development, effective periods of fungicide control and grain yields of maize hybrids, (Planted 6 Dec 1991) for fungicide treatments applied at different dates after planting (DAP)(a)

Fungicide application details		Disease severity (%)(c)					S AUDPC(d)	r(e)	R^{2}(f)	Fungicide effective period		Grain yield
Date applied	DAP	Disease(b) at treatment date	Assessment (DAP)							Length(g) in days	End of effective period (DAP)	Kg/ha^{-1}
			86	99	116	140						
RS 5206(h)												
Non-sprayed	-		8	55 a(i)	88 a(i)	100 a(i)	68 a(i)	13.1 a(i)	0.977	-		4101 c(i)
28/1	53	0	1	33 b	73 b	99 a	58 b	15.9 a	0.961	14 c(i)	63	5524 b
11/2	67	1.5	2	1 c	5 e	69 bc	19 c	9.8 b	0.827	39 a	104	7929 a
21/2	78	5.7	5	18 c	16 d	83 ab	28 d	8.8 b	0.878	26 b	104	7770 a
6/3	90	21.5	10	42 b	35 c	63 c	34 d	4.5 c	0.752	17 c	107	7075 a
							42(j)					6480(k)
CRN 4526(h)												
Non-sprayed	-		3	49 d	80 f	100 d	63 d	14.1 e	0.966	-		3463 f
28/1	53	0	1	14 f	56 g	100 d	50 c	16.6 f	0.997	15 e	59	5177 e
11/2	67	0.5	2	1 g	3 i	64 e	16 b	8.8 h	0.879	32 d	99	6551 d
21/2	78	2.1	2	4 g	10 i	71 e	21 ab	8.8 gh	0.976	25 d	104	6364 d
6/3	90	9.8	6	35 e	25 h	56 e	27 a	4.6 g	0.846	17 e	107	5387 e
							36(j)					5384(k)
Mean												
SE (of a mean)				3.0	3.6	5.3	2.5	1.6		2.4		280.6
Grand mean				23.9	39.0	80.4	38.6	11.4		23.1		5932
CV%				13.6	7.8	5.5	5.4	26.8		16.0		8.7

(a)Days after planting.
(b)Disease severity from logistic progress curves.
(c)Disease severity is percent leaf tissue with symptoms of GLS.
(d)Standardised area under disease pressure curve.
(e)Infection rate x 100, calculated by regressing the logistic transformation on time (DAP).
(f)Coefficient of determination.
(g)Calculated from logistic progress curves.
(h)Disease first observed 60 DAP, anthesis occurred 77 DAP and physiological maturity at 150 DAP.
(i)Means within a column having letters in common do not differ significantly P($\leq$0.05) Duncan's multiple range test.
(j)AUDPC values for RS 5206 and CRN 4526 were significantly (P<0.001) different.
(k)Grain yield of RS 5206 was significantly higher than CRN 4526 (P<0.001).

1992/93 Season. Despite unfavourable weather conditions for disease during the vegetative growth stage of the crop, GLS symptoms were observed before anthesis, 60 DAP. Initial disease development was slow, with disease in non-sprayed treatment rising to 7,8% severity 36 days after first symptoms were observed. Subsequent progress was rapid, increasing to 60% severity in the following 21 days (Table 4).

All fungicide treatments provided significant reduction in disease (AUDPC) and reduced the infection rate (Table 4). Fungicides provided effective control of disease up to 118 DAP, but did not provide control through to physiological maturity at 149 DAP. Disease progress following the FEP progressed normally and parallelled the non-sprayed treatment (Fig 2A and 2B). Multiple spray treatments resulted in longer FEP (41 to 52 days) than single spray treatments (26 to 33 days). The spray-treatment initiated 83 DAP; when the basal five-leaves of the host were symptomatic provided the longest FEP (33 days) of the single spray-treatments.

able 4. Gray leaf spot (GLS) disease development, effective periods of fungicide control and grain yields for fungicide treatments applied at different stages of disease development on the host (RS 5206, 1992/93 season).

Fungicide application details				Disease severity %(c)						STD(d) AUDPC	r(e)	R^2(f)	Effective period		Grain yield
eat- ent no.	Dates applied	DAP(a)	Host stage for disease symptoms for first spray	Disease(b) at treatment date	Assessment (DAP)								Length(g) in days	End of effective period DAP	Kg/ ha
					83	96	112	117	145						
	Unsprayed		Unsprayed(i)		3.4	7.8	21.4 a(j)	60.0 a(j)	95.0 a	37 a	9.8 a	0.983			5060
	11/1	67	Basal 1-2 leaves	0.8	1.0	1.0	3.4 c	11.1 b	47.5 bc	10 bc	5.6 b	0.967	29 a	96	5830
	27/1	83	Basal 5-leaves	3.6	3.6	8.1	8.6 b	8.9 b	53.7 b	12 b	5.7 b	0.933	33 ab	116	6550
	10/2	97	Up to ear height	5.0	4.8	5.0	5.6 bc	7.5 b	28.8 bc	9 b	3.9 b	0.904	26 a	123	6190
	11/1, 27/1	67, 83	Basal 1-2 leaves	0.8, 1.1	1.1	1.3	1.8 c	3.2 b	30.6 bc	6 cd	4.3 b	0.932	41 b	108	6370
	11/1, 10/2	67, 97	Basal 1-2 leaves	0.8, 1.3	1.1	1.3	1.4 c	2.7 b	29.4 bc	6 cd	4.3 b	0.905	49 c	116	5870
	27/1, 10/2	83, 97	Basal 5-leaves	4.0, 4.5	4.3	4.4	5.6 bc	5.7 b	23.1 c	8 bcd	3.8 b	0.902	49 c	132	6370
	11/1, 27/1, 10/2	67, 83, 97	Basal 1-2 leaves	0.8, 0.9, 1.0	0.8	0.9	1.1 c	1.9 b	18.8 c	4 d	3.8 b	0.915	52 c	119	7080
(of a mean)							1.7	3.0	8.8	1.9	0.7		3.6		407.2
and mean							5.7	12.6	40.9	11.9	5.1		39.9		6170
%							61.3	47.6	43.0	32.5	27.1		18.1		13.2

DAP = days after planting
Disease severity estimated from logistic progress curves
Disease severity is percent leaf tissue with symptoms of GLS
Standardised area under disease progress curve
Infection rate x 100, calculated by regressing the logistic transformation on time (DAP)
Coefficient of determination
Calculated from logistic progress curves
Average grain yield of fungicide sprayed treatments was significantly higher than non-sprayed control ($P<0.08$) Duncan's multiple range test
Disease first observed 60 DAP, anthesis occurred 80 DAP and physiological maturity at 149 DAP
Means within a column having letters in common do not differ significantly ($P<0.05$) Duncan's multiple range test

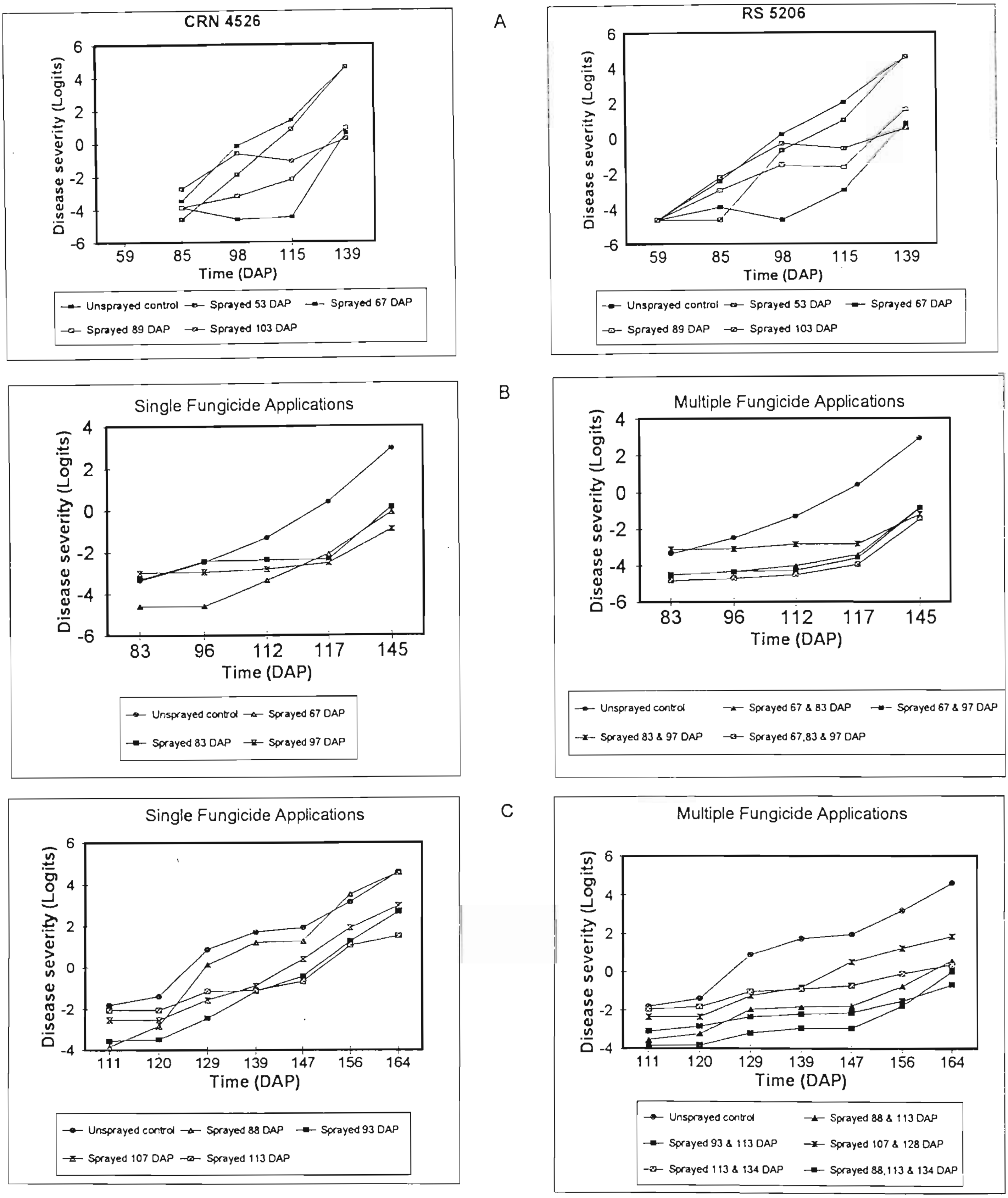

Figure 2 Logistic means of disease progress of gray leaf spot disease severity of fungicide treatments applied at different times of application against time for: A 1991/92, B 1992/93 and C 1993/94

1993/94 Season. Climatic conditions were favorable for GLS and disease was first observed earlier (53 DAP) than in the previous season (60 DAP). Disease progress followed a similar trend to that in previous seasons, with initial slow disease progress (up to 2% disease in 40 days) followed by rapid progress reaching 71% severity in the following 36 days.

All fungicide treatments reduced disease severity, by 147 DAP, shortly before the crop was physiologically mature. Fungicides also reduced overall disease (AUDPC, Table 5), when all treatments, except single spray treatment 2, (beginning 88 DAP when lesions were present on the basal two-leaves), reduced disease to levels lower than the non-sprayed treatment. The reduction in disease by fungicide treatment was reflected in significant grain yield responses.

The time of application and number of spray treatments under conditions favorable for disease development in 1993/94 contributed to large differences in disease severity, the length of the FEP and the grain yields harvested. The three-spray and two-spray programs, (except for treatment 8, beginning at higher initial disease severity, 107 DAP), provided significantly better control of disease (AUDPC) and longer FEP than the single-spray and non-sprayed treatments. This improved disease control was also reflected in the higher grain yields achieved by these treatments.

The spray treatment 2, (beginning 93 DAP when disease was 2% on the basal five-leaves), provided the lowest disease (AUDPC = 32) of the single spray treatments, and had the longest FEP (29 days), although this was not reflected in higher grain yields. The treatment (7) initiated 93 DAP, with lesions on basal five-leaves, also had the lowest disease (AUDPC = 9), the longest FEP (54 days) and amongst the highest grain yields of the two-spray treatments. The three-spray program provided similar control of disease (AUDPC) to treatment 7. Although the three-spray treatment provided the longest FEP, it was not significantly different to treatment 7. The three-sprays also produced the highest grain yield but was not significantly higher than other two-spray treatments excepting treatment 8.

Only the multiple spray treatment and treatment 5 reduced infection rates. The remaining single spray treatments had infection rates similar or higher than the non-sprayed treatment.

able 5. Gray leaf spot (GLS) disease development, effective periods of fungicide control and grain yields for fungicide treatments applied at different stages of disease development on the host (RS 5206, 1993/94 season).

Fungicide application details					Disease severity %(c)							STD(d) AUDPC	r(e)	R^2(f)	Effective period		Grain yield
atment No.	Dates applied	DAP(a)	Host stage for disease symptoms for first spray	Disease(b) at treatment date	Date assessed DAP										Length(g) in days	DAP at end of effective period	Kg/ha
					111	120	129	139	147	156	164						
			Non-sprayed	-	14.1	20.0	71.3	85.0	87.5 a(h)	96.0 a(h)	99.0 a(h)	69 a(h)	12.1 b(h)	0.98	-	-	2266 a*(h)
	22/1	88	Basal 2-leaves(i)	1.5	2.1	5.6	53.8	77.5	78.8 b	97.2 b	99.0 a	60 a	16.0 a	0.98	22 fg(h)	110	3141 ab
	27/1	93	Basal 5-leaves	2.0	2.8	3.0	8.1	24.4	40.0 c	78.7 c	93.7 ab	32 cd	12.5 b	0.98	29 ef	122	4211 bc
	10/2	107	Up to ear-height	10.0	7.5	7.5	17.5	29.4	39.4 c	87.5 c	95.2 ab	38 b	11.0 b	0.95	18 gh	125	4033 bc
	16/2	113	Above ear-height	14.0	11.3	11.3	24,4	25.0	33.8 c	75.0 c	82.5 b	35 bc	7.4 cd	0.94	13 h	126	4971 c
	22/1, 16/2	88, 113	Basal 2-leaves	1.5, 3.1	2.8	3.8	12.5	13.8	14.4 d	31.9 d	63.7 c	17 e	7.3 cd	0.96	36 de	124	6525 d
	27/1, 16/2	93, 113	Basal 5-leaves	2.0, 2.0	2.1	2.1	3.9	5.0	5.0 e	14.4 e	50.0 d	9 f	6.5 d	0.89	54 b	147	6600 d
	10/2, 3/3	107, 128	Up to ear height	10.0, 20.0	8.8	8.8	22.5	30.6	37.5 c	77.5 c	86.2 ab	37 bc	8.4 c	0.97	38 cd	145	4389 bc
	16/2, 9/3	113, 134	Above ear height	14.0, 26.0	12.5	13.8	26.3	28.8	32.5 c	46.9 c	58.7 cd	30 d	4.4 e	0.98	44 c	157	6235 d
	22/1, 16/2, 9/3	88, 113, 134	Basal 2-leaves	1.5, 3.0, 9.0	4.3	5.5	8.8	10.0	10.6 de	18.1 de	33.7 e	12 f	4.1 e	0.95	68 a	156	6957 d
(of a mean)									2.9	4.6	4.5	1.5	0.5		2.6		814.8
nd mean									37.9	62.3	76.4	34	9.0		36.0		4933
%									15.5	14.7	11.7	9.1	11.9		14.3		16.5

DAP = days after planting

Disease severity estimated from logistic progress curves

Disease severity is percent leaf tissue with symptoms of GLS

Standardised area under disease progress curve

Infection rate x 100, calculated by regressing the logistic transformation on time (DAP)

Coefficient of determination

Calculated from logistic progress curves

Means within a column having letters in common do not differ statistically ($P \leq 0.05$) Duncan's multiple range test

Disease first observed 53 DAP, anthesis occurred 78 DAP and physiological maturity at 153 DAP

DISCUSSION

Farmers in KwaZulu-Natal receive approximately US\$139 ton^{-1} for maize. Farmers who normally harvest 5 tons of grain ha^{-1} can lose between 1 and 3 tons grain yield ha^{-1} from GLS. The cost of fungicide is US\$25 ha $^{-1}$ and the cost of aerial application is US\$11 ha^{-1}, making a total cost for control of US\$36 ha^{-1}. The break-even increase in grain yield is therefore 259 kg ha^{-1}. Responses to single spray treatments varied between 875 and 2705 kg ha^{-1}, to two sprays were 1310 and 4334 kg ha^{-1} and to three sprays were between 2020 and 4691 kg ha^{-1}. Cost of fungicide treatments were therefore economically justified.

Yield is a function of photosynthesis carried out by the plant and is related to the leaf area and its duration after flowering (5). The top eight or nine leaves contribute 75 to 90% to grainfill (1). Further, the number of kernels on the ear are established at anthesis (9). It is therefore important to maintain the upper leaves in a healthy condition until physiological maturity, if maximum yields are to be achieved. Foliar diseases such as GLS, under favourable conditions, can result in extensive leaf blighting, leading to loss in grain yield. The loss in photosynthetic area also causes depletion of carbohydrate from the stalk and roots in an attempt by the plant to meet the demands of grain filling, and predisposes the plants to early root senescence and stalk-lodging, exacerbating the yield losses due to GLS (11).

In the four seasons of studies at CADI, GLS was observed in the crop before anthesis, but as disease development was initially slow, blighting of upper leaves was post-anthesis. The main effect of blighting on yield was a reduction in grainfill rather than a reduction in the number of kernels that are determined at anthesis.

As the ear is the dominant sink of the post-anthesis maize plant the loss of photosynthetic area by blighting causes photosynthate to be diverted from the stalks and roots at greater than normal levels causing them to senesce prematurely. The result is not only a loss in grain yield but significant losses due to premature root death, stalk necrosis, stalk lodging and death of the plant (11). The trial consistently indicated that fungicide treatments were effective in delaying the leaf blighting process and those treatments with the longest FEP usually yielded the highest grain yield.

Time and frequency of application of fungicide treatments were critical to the length of the FEP. It is customary to begin a fungicide program as early as possible after detectable levels of disease develop. With the characteristic slow initial development of GLS disease, it is not easy to define the stage of "detectable level" of disease when spraying should commence. Certainly the 1991/92 studies indicated that spray applications beginning before GLS was observed were less effective, whilst applications made after the pathogen had begun to rapidly increase were also less effective in controlling the epidemic. These early experiments indicated that treatment should be initiated after the disease was observed but before high levels were present. Subsequent trials confirmed these this conclusion.

Spray applications initiated when disease had progressed to the basal five leaves of maize usually provided the longest FEP's and highest grain yields of the single spray treatments, but FEP's were of insufficient duration to protect the crop until it was physiologically mature. The multiple-spray treatments, with a longer combined FEP, delayed the development of disease longer, and resulted in better disease control and higher grain yields than single spray treatments. Further, the number of basal leaves affected when the fungicide treatment was initiated played an important role in determining the length of control. This point is clearly shown in the 1993/94 data, when two well timed sprays, commencing when disease was 2% and present on the basal five leaves, provided as effective control of disease as the three-spray program commencing at lower disease ratings withg disease present on the basal two leaves. The difference in yield between the two treatments, was not significant ($P \leq 0.05$).

The importance of controlling disease through to physiological maturity was demonstrated. The relatively long period after breakdown in fungicide control until physiological maturity in the three spray program in 1992/93 resulted in a relatively small increase (29%) in grain yield. This long period after fungicide breakdown may explain the lack of significance between treatments in grain yield, in spite of significant differences in control of disease (AUDPC). In contrast, the three- spray treatment in 1993/94 provided effective disease control until after physiological maturity and resulted in a 68% increase in grain yield. The object, therefore, of any spray program to delay disease in maize through to physiological maturity.

The trials determined the most favorable time to commence fungicide treatment i.e. approximately 1 to 2% leaf blighting, depending on hybrid susceptibility, and when lesions are visible on the basal five leaves of the plant. Further sprays may be necessary to provide control until the crop is physiologically mature. Spray intervals depend on duration of weather conditions favourable for disease, and results from trials indicate that this interval may vary between 20 and 30 days.

Yield responses to fungicide treatments appear to be a function of the growth stage when sprays are initiated, the amount of disease at spray date, the FEP and control through to physiological maturity.

Experiments were conducted with the GLS-susceptible hybrid RS 5206. Resistance appears to be of a rate-reducing type, and less susceptible hybrids may react differently over the timing and number of spray applications. Finally, the trials were treated with equipment providing full-cover spray application up to 450 L ha^{-1}. Such medium volume applications may not be feasible in commercial agriculture and more research is required on different methods of application and spray volumes, including low-volume aerial application.

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CHAPTER 7

THE ECONOMIC BENEFITS OF FUNGICIDE TREATMENT OF MAIZE FOR THE CONTROL OF GREY LEAF SPOT (*CERCOSPORA ZEAE-MAYDIS*) IN KWAZULU-NATAL(1)7

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Grey leaf spot (*Cercospora zeae-maydis*, Tehon and E.Y. Daniels) is a relatively new disease of maize in South Africa. It is capable of reducing grain yields by 20 to 60% in KwaZulu-Natal. Fungicides are widely used for control, but must be applied at the correct stage of disease development. Repeated applications may be necessary for effective control to be achieved. There is a paucity of information on the economic justification of these control measures. The aim of this study was to estimate the economic benefits of single and multiple sprays applied to maize at different stages of disease development. The economic analysis was based on the average operating costs of 18 dryland maize farms in

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the Winterton and Karkloof areas, where fungicides have been used to control the disease. These costs were used in the analysis of data generated from trials at Cedara to determine the most effective times and frequency of fungicide application for the control of grey leaf spot. Triple spray treatments under both high and low disease levels provided better control of disease (higher yield gains over non-sprayed treatment) but did not always give highest added profit ha^{-1}. The single-spray treatment at the five-leaf stage of infection however, provided the highest added profit ha^{-1} in 1992/93, whilst, under high disease levels in 1993/94, the triple-spray treatment resulted in the highest added profit. Least risk-averse farmers would probably select the triple-spray programme under high levels of disease, since it provided highest added profit ha^{-1}. The double-spray programme, with lower added profit ha^{-1} under high levels of disease, may, however, be preferred by more risk-averse individuals. Under low levels of disease, the single-spray treatment giving higher added profit ha^{-1} may be more attractive to least risk-averse farmers.

Keywords: *Cercospora zeae-maydis*, Grey leaf spot disease, fungicides, financial benefits

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Introduction

Maize (*Zea mays* L.) accounts for 36% of all crops grown in South Africa. In 1993/94, 3,9 million ha were planted, with a marketable value of R4 128 million. Of this, 2,75 Mt were sold for human consumption, 6,47 Mt for animal fodder and 2,75 Mt were exported (Anonymous, 1995). The extent to which weeds, insect pests and diseases contribute to losses in yield and grain quality has not been quantified (Chambers, 1986). Gevers, Lake and McNab (1990) forecast that *Cercospora zeae-maydis* Tehon and E.Y. Daniels, the causal fungus of grey leaf spot (GLS), and at the time a minor disease, could assume greater importance than previous disease epidemics. In 1994, Ward and Nowell (1997) reported that the disease had spread throughout the maize growing areas of KwaZulu-Natal and that it was capable of reducing grain yields by as much as 60%.

Fungicides are a major control measure of many crop diseases, and are applied aerially on commercial maize in South Africa (Rijkenberg, 1997; Ward & Nowell, 1997). The timing and frequency of fungicide applications are critical for successful control of GLS (Ward, Laing & Rijkenberg, 1997). In the United States, chemical control of maize diseases has been of benefit on high value crops such as maize hybrid seed, but chemical control has not been practical on commercial maize crops (Coates & White, 1995).

In South Africa the application of fungicides to commercial maize crops has been effective in controlling GLS, but there is a paucity of information on the economic justification of such control measures. Cost of disease control and the value of preventable crop losses are components to consider in disease control decisions.

Other factors influencing such decisions are frequency and timing of treatments and efficacy of control measures (Wegulo, 1994).

The objectives of this study were to establish most effective times for fungicide applications and the frequency of applications necessary to achieve effective control of GLS. These were identified by estimating the economic benefits of using single and multiple applications of fungicides to control GLS. Economic benefits consist of the extra (marginal) income from additional yield due to GLS control, less the increased (variable) costs of fungicide use.

Materials and Methods

Field Data

The data in this study were generated from an investigation conducted in the 1992/93 and 1993/94 seasons at Cedara Agricultural Development Institute (CADI) at Cedara (29°32'S, 30°17'E, alt 1070 m) (Ward *et al.*, 1996). The trial area had been continuously cropped to maize for the previous 10 years, and the trial was conducted on the same field during both the 1992/93 and 1993/94 seasons. Weather conditions varied over the two seasons. Hot, dry weather prevailed during 1992/93, with only 63% of the average annual rainfall recorded during the growing season. In contrast, the rainfall in 1993/94 was above average and well distributed throughout the growing season. Temperatures were lower than average, and mists were frequent, especially in January and February 1994, during grainfill.

The GLS susceptible hybrid RS 5206 was no-till planted into GLS infected maize debris from the previous season, in late October 1992 and in early November 1993. A randomised complete blocks design with three replications was used. Each plot consisted of 8 rows spaced 0,75 m apart and 12 m in length, resulting in 45 000 plants ha^{-1}. Fertilizer applied was sufficient for an eight-ton ha^{-1} grain crop. Normal weed and insect pest control practices for the area were followed.

Treatments comprised different fungicide application times at different stages of GLS development, using single- and multiple-spray applications. Spray treatments were initiated when disease lesions were observed on the basal two-leaves, the basal five-leaves, and all basal leaves to ear-height. A fungicide containing 187,50 g carbendazim and 93,75 g flusilazole (Punch Xtra, Du Pont De Nemoirs and Coy) was applied in a spray volume of 450 L ha^{-1}. The spray solution was applied with a CO_2 pressurised back-pack sprayer having a vertically mounted spray boom on which three Whirlrain ¼" WRW 2 - 20° spray-nozzles were mounted one metre apart. Fungicide sprays were applied to the central four-rows of each plot. The central 10 m of the two middle-rows were assessed for disease development and hand-harvested for grain yields. (Ward *et al.*, 1996).

Disease assessments based on standard whole plant assessment diagrams (Ward *et al.*, 1996), were used as a guide to estimate percentage disease severity. Assessments were made throughout grainfill at 10- to 14-day intervals. These data were used to calculate area under disease progress curve (AUDPC), which is a summary of the disease epidemic (Berger, 1987). The AUDPC was standardised (SAUDPC) by dividing the AUDPC value by the duration of the epidemic, to enable comparisons to be made from one season to another (Fry, 1978). Grain yields were expressed in kg ha^{-1} at 12,5% moisture.

Statistical analysis

The SAUDPC and grain yield data were analysed by analysis of variance (ANOVA). Mean separations were based on Duncan's Multiple Range Test at the 5% level of probability. The analysis was conducted using Genstat 5.2 (Anon., 1987). Ninety five percent confidence limits were calculated for the difference in yield between each fungicide treatment and non-sprayed treatment means in order to establish upper and lower confidence limits for added yield due to fungicide treatment. These added yield upper and lower limits were converted to added profit upper and lower limits by multiplying the yield limits by the expected average maize price (R400 ton^{-1}), and subtracting the added costs of fungicide treatment and harvesting of the yield limits (Saville, 1983).

Economic analysis

Costs for the economic analysis were based on the average operating costs from a survey of 18 representative dryland maize farms in the Winterton and Karkloof areas of KwaZulu-Natal. Selection of farms was based on the presence of GLS disease and use of fungicides for control during the 1993/94 season. Costs that were common to all treatments were regarded as fixed costs. These included machinery and labour for land preparation, planting, fertilization, insecticide and herbicide application, and the costs of fertilizer, seed and agro-chemicals. Interest, depreciation, fuel, repairs and insurance were included in machinery costs. Additional costs for fungicide, fungicide application, and harvesting of increased yield resulting from fungicide usage were defined as variable costs (see Table 1). The expected average maize price of R400 ton^{-1} was used to estimate the value of preventable crop loss.

The gain in yield (G) due to fungicide treatment is the difference between yield with fungicide treatment (Yc) and yield of the non-sprayed treatment (Yo), as shown in equation (i):

$$G = Yc - Yo \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (i)$$

The added profit due to fungicide treatment (Pa) was calculated from the gain in yield (G) multiplied by the maize price ton^{-1} (R) less the costs of fungicide (F), fungicide application (A) and the extra cost of harvesting the gain in yield (H), as shown in equation (ii):

$$Pa = (G \times R) - (F + A + H) \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (ii)$$

Added profit (Pa) reflects the estimated economic benefits of fungicide use as it shows the extra income less increased costs associated with fungicide treatment.

Table 1. Variable and fixed costs of production of maize in Winterton and Karkloof in 1993/94 (18 farms)

Item		Rand ha^{-1}
Fixed Costs		
Machinery and labour		292,52
Seed		104,00
Fertilizer		474,00
Insecticides and herbicides		225,40
		1 095,92
Variable costs		
Harvesting ton^{-1} (machinery and labour)		26,12
Fungicide	- chemical	75,50
	- application	42,50
		144,12

Results

Disease severity and grain yields

Grey leaf spot disease severity and grain yields affected by frequency and timing of fungicide treatments have been reported elsewhere (Ward *et al.*, 1997). Only a summary of results necessary for the economic analysis has been presented. Weather conditions varied over the two seasons. The 1992/93 season was hot and dry and unfavourable for GLS development. In contrast, the 1993/94 season experienced above-average and well distributed rainfall, which was favourable for the disease.

1992/93 Season

Although conditions for GLS development were unfavourable, disease was observed before anthesis, but remained at relatively low levels, shown by low SAUDPC levels (Table 2). All fungicide treatments provided effective control of diseases (SAUDPC) but did not control disease through to physiological maturity (Ward *et al.*, 1996). Treatment at the five-leaf stage of lesion development provided the longest period of fungicide control for the single-spray treatments (Ward *et al.*, 1996). The triple-spray programme, double-spray treatments applied at 67/83 days after planting (DAP) and 83/97 DAP and the single-spray at the five-leaf stage provided significantly higher grain yields gains than the non-sprayed treatment, using the least significant difference (LSD) method of comparison. Grain yield gain over the non-sprayed treatment for these treatments all exceeded the LSD of 1.21 t ha^{-1} at the 5% level of probability.

Table 2. Grain yield (t ha^{-1}) and added profit (R ha^{-1}) for time and frequency of fungicide spray treatments, 1992/93.

Fungicide treatment			Grain yield (t ha^{-1})				Added profit[d] (R ha^{-1})		
				Yield gain over non-sprayed					
Application date [a]	Host stage of lesion development at first spray	SAUDPC [b]	Actual	Actual	Lower [c] limit	Upper[c] limit	Actual	Lower limit	Upper limit
67, 83, 97	Basal 2 leaves	4 d[e]	7.08	2.02	0.82	3.22	401	(47)	850
83	Basal 5 leaves	12 b	6.55	1.49	0.29	2.69	439	(10)	888
67, 83	Basal 2 leaves	6 cd	6.37	1.31	0.11	2.51	254	(195)	702
83, 97	Basal 5 leaves	8 bcd	6.37	1.31	0.11	2.51	254	(195)	702
97	Up to ear height	9 b	6.19	1.13	(0.07)	2.33	304	(144)	753
67, 97	Basal 2 leaves	6 cd	5.87	0.81	(0.39)	2.01	67	(382)	516
67	Basal 2 leaves	10 bc	5.83	0.77	(0.43)	1.97	170	(279)	619
Non-sprayed		37 a	5.06	---			---		
SE (of mean)		1.9	0.401						
CV (%)		32.5	13.2						
SE (of difference)				0.567					
LSD (5%)				1.21					

Application date measured in days after planting

SAUDPC is the area under disease progress curve, standardised by dividing AUDPC by the time duration (days)

Lower- and upper-gain in yield were calculated from 95% confidence limits

Added profit equals gain in yield multiplied by maize price (R400 ton^{-1}) less costs of fungicides, fungicide application and harvest of yield gain

Means within a column having letters in common do not differ significantly by Duncan's multiple range test (R<0.05)

1993/94 season

A higher level of disease during 1993/94 adversely affected grain yields. All fungicide treatments controlled disease through to physiological maturity (Ward *et al.*, 1996). Significant differences in disease severity and grain yields were associated with different times of application and the number of fungicide treatments applied (Table 3). The triple-spray and double-spray treatments, except for the double-spray treatment commencing when disease was present on basal-leaves up to ear height, controlled disease significantly better than the single and unsprayed treatments. All fungicide treatments, except the single spray 88 DAP, increased grain yields significantly more than the non-sprayed control. The multiple-spray treatments, except for the double-spray treatment when disease was already up-to-ear height, provided higher grain yields than the single-spray treatments.

ble 3. Grain yield (t ha^{-1}) and added profit (R ha^{-1}) for time and frequency of fungicide spray treatments, 1993/94.

Fungicide treatment			Grain yield (t ha^{-1})				Added profit$^{(d)}$ (R ha^{-1})		
				Yield gain over non-sprayed					
Application date $^{(a)}$	Host stage of lesion development at first spray	SAUDPC $^{(b)}$	Actual	Actual	Lower $^{(c)}$ limit	Upper$^{(c)}$ limit	Actual	Lower limit	Upper limit
3, 113, 134	Basal 2 leaves	12 f$^{(e)}$	6.96 d$^{(e)}$	4.69 1.18	3.51	5.87	1 400	958	1 842
3, 113	Basal 5 leaves	9 f	6.60 d	4.33	3.15	5.52	1 384	942	1 826
3, 113	Basal 2 leaves	17 e	6.53 d	4.26	3.08	5.44	1 356	914	1 798
)7, 128	Up to ear height	37 bc	4.39 bc	2.12	0.94	3.31	558	116	1 000
3	Basal 5 leaves	32 cd	4.21 bc	1.95	0.76	3.13	609	167	1 051
)7	Up to ear height	38 b	4.03 bc	1.77	0.59	2.95	543	101	985
3	Basal 2 leaves	60 a	3.13 ab	0.87	(0.32)	2.04	205	(264)	647
on-sprayed		69 a	2.27 a						
Ξ (of mean)		1.5	0.407						
√ (%)		9.1	16.5						
Ξ (of difference)				0.576					

Application date measured in days after planting

SAUDPC is the area under disease progress curve, standardised by dividing AUDPC by the time duration (days)

Lower limit and upper limit gain in yield were calculated from 95% confidence limits

Added profit was calculated from gain in yield multiplied by maize price (R400 ton^{-1}) less the costs of fungicides, fungicide application and harvest of yield gain

Means within a column having letters in common do not differ significantly by Duncan's multiple range test ($R<0.05$)

Economic analysis

Average production costs of maize for the 18 dryland farms in Winterton and Karkloof in 1993/94 (Table 1) show that fixed costs ha^{-1} averaged R1 095,92, while variable costs ha^{-1} associated with harvesting and fungicide use averaged R144,12. These data and the expected maize price of R400 ton^{-1} were substituted into equations (i) and (ii) to obtain the added profit ha^{-1} data in Tables 1 and 2.

1992/93 season

All fungicide treatments increased profit ha^{-1} compared to the non-sprayed treatment (Table 2). However, based on the added yield LSD analysis, only the triple-spray treatment, single-spray treatment at the five-leaf stage of infection, double-spray treatment at 67/83 DAP and double-spray treatment at 83/97 DAP significantly increased profit ha^{-1}. Added profit ha^{-1} was highest for the single spray treatment (R439) which also had the highest upper limit profit ha^{-1} (R888) and smallest added profit ha^{-1} lower limit (negative R10).

1993/94 season

Higher disease levels during 1993/94 led to greater potential payoff from fungicide treatment, as shown by higher actual added profit ha^{-1} compared to the 1992/93 season (R205 to R1400 compared with R170 to R401, Tables 2 and 3). Added profit ha^{-1} for each treatment was statistically significant compared to the non-sprayed treatment. The triple-spray treatment at the two-leaf stage gave the highest added profit ha^{-1} (R1400), while only the single-spray application at the two-leaf stage had a negative lower limit for added profit ha^{-1}. These results indicate the potential economic benefits from fungicide application, particularly under higher disease severity.

Discussion

Additional grain yields due to fungicide use in 1992/93 for single-sprays varied from 770 to 1 490 g ha^{-1}, for double-sprays from 810 to 1 310 kg ha^{-1} and for triple-sprays it was 2 020 kg ha^{-1}. In 1993/94 the additional grain yields ranged from 870 to 1 950 kg for single-sprays, 2 120 to 4 330 kg for double-sprays and it was 4 690 kg for the triple-spray treatment. An economic assessment of the estimated value of these yield responses relative to fungicide costs was therefore made to ascertain the relative economic benefits of these treatments.

There was less disease in the dry season of 1992/93 than in 1993/94 when normal well-distributed rains occurred. The gain in yield due to fungicide treatment in the dry season was lower than in the wet season. Hence the added yield gain from the triple-spray treatment was not significantly higher than a well-timed single-spray treatment in 1992/93 (LSD 1,21, $P<0.05$, Table 2). In the wet 1993/94 season, with more disease, multiple spray treatments added more maize yield than single-spray treatments. These results are relevant in areas where there are no commercial hybrids resistant to GLS, and alternative measures of control such as crop rotations and tillage practices have only limited effectiveness (Ward & Nowell, 1997). The success in controlling GLS using fungicides is, however, dependent on the correct timing and frequency of the fungicide treatment (Ward *et al.*, 1997).

Wegulo (1994) suggested that the optimum time to start a fungicide programme is as early as possible after detectable levels of GLS develop. Results from this study, however, show that fungicide treatments starting when lesion development is first visible on the basal two-leaves did not always provide most effective control or highest added grain yields. In the wet 1993/94 season, with high disease levels, the single and double sprays, when disease was present on the basal five-leaves, provided effective control of GLS. Treatments earlier or later provided less effective control. The multiple spray treatments, except for the double-spray treatment when disease had developed to ear height, provided the most effective control of GLS and the highest added grain yield ha^{-1}. The triple-spray programme in both seasons gave the highest added grain yield ha^{-1}, but economic analysis indicated that this was not always the highest added profit ha^{-1} treatment.

The decision to apply fungicides for control of GLS to increase maize yields is based on the expectation that financial return from investment in fungicide treatment will exceed cost of treatment. The outcome of such decisions cannot be predicted with confidence, as farmers face uncertainty due to maize producer price policy changes (domestic maize price is no longer based on production costs, but rather on market supply and demand), rising and variable input costs, weather conditions (drought or hail following fungicide treatment during grainfill can negate the beneficial effects expected from treatment). Availability of finance, managerial-ability and the risk attitude of the individual farmer will also influence the decision to apply fungicides. Most farmers are probably risk-averse, requiring compensation for taking risks (for example, applying fungicides for potential added profit), with the required compensation increasing as the risks and/or levels of risk aversion increase (Barry, Hopkins and Baker, 1983).

There is a trade-off between risk and expected profit, and the higher the expected profit, the higher is the risk (Barry, Hopkins & Baker, 1983). The dilemma facing the farmer in his decision to apply fungicides, and the number of applications necessary to effect control, is whether the expected added income due to fungicide treatment will exceed the added costs of fungicide, fungicide application and harvesting of added yield. Fungicide spray applications also imply higher input costs and hence place more capital at risk. This was well illustrated in 1993/94, under high levels of disease, for the single, poorly-timed (two-leaf stage) spray which has a lower limit yield and added profit loss ha^{-1}. In contrast, with lower levels of disease in 1992/93, a single well-timed spray (five-leaf stage) gave the highest added profit ha^{-1}, whilst the more costly triple-spray treatment had the highest added yield but a lower profit ha^{-1}. Farmers will base their decisions to apply fungicide on their expectations of added yield, maize prices and variable input costs. An experienced farmer will base his expectation of crop yield potential on its vegetative growth, and the potential for GLS from prevailing weather conditions. As farmers are generally risk-averse, they will only apply fungicides if the expected profit ha^{-1} from increased yields adequately compensates them for their individual subjective assessment of the risks associated with spraying. They will select or modify spray programmes, such as those reported in this paper, to suit their individual situations.

The expected range of added profit ha $^{-1}$ from fungicide treatment is a useful parameter to justify spraying for the control of GLS to increase grain yields. For example, in 1992/93 the added profit ha^{-1} ranges (negative R382 to R888) indicate the potential gains and losses ha^{-1} for spray treatments. Individual farmers need to assess for themselves whether the potential gains relative to potential losses justify treatment application (see Discussion, which provides information on the potential range of returns that can be expected from the increased costs). Under the (wet) 1993/94 season conditions (higher risk due to higher potential payoff from fungicide use), less risk-averse individuals, prepared to pay more to achieve a higher expected added profit, would likely select the triple-spray or double-spray treatment at 93/113 DAP. More risk-averse farmers wanting to maintain relatively high added profits ha^{-1} with lower input costs would probably select a double-spray programme at 101/128 DAP, or single-spray applications at 93 or 107 DAP.

The situation in the dry season 1992/93, under lower disease levels, differed from 1993/94. The triple-spray programme with the highest added grain yields was not the most profitable. The single-spray treatment initiated at the 5-leaf stage of infection, with lower added grain yields and lower input costs, had the highest added profit ha^{-1}. Under these conditions this would probably have been the spray programme selected by least risk-averse farmers.

The analysis of data was conducted on trial data generated from the GLS-susceptible hybrid RS 5206. The added profits may vary with differing yield responses from other hybrids, but the principles used to establish the parameters to establish the economic benefits of fungicide treatment remains the same.

Conclusion

The decision to apply fungicides for the control of GLS is justified in areas where the disease is epidemic. Highest added maize yield response ha^{-1} is not necessarily the best parameter justifying treatment. Rather the economic implications of each treatment should be considered, namely the *expected* added income compared with the *expected* added costs of fungicide treatment. Individual farmers will subjectively compare the *expected* added profit ha^{-1} (added income minus added costs) with the potential variability in expected added profit ha^{-1} (upper and lower limits to added profit ha^{-1}) of each treatment when deciding which (if any) fungicide treatment to use. Expected added profit ha^{-1} levels will change over time, depending on season and relative changes in output and input prices.

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CHAPTER 8

GENERAL DISCUSSION

The sudden increase in the prevalence and severity of GLS in South Africa since 1988 has caused severe annual yield losses to maize producers, especially in the province of KwaZulu-Natal. The original research on the disease in South Africa was initiated by Ward & Nowell (1994) who established that yield losses to GLS varied between 30 and 60%, depending on hybrid susceptibility and weather conditions. These high yield losses are attributed to genetic vulnerability of hybrids, high inoculum levels and the long periods during which leaf-blighting occurs during grain-fill. Stromberg & Donahue (1986) suggested maturity group might be an important factor affecting the disease. Long-season hybrids with higher yield potentials are at greater risk from GLS, as they are subjected to blighting for a longer time during the grain filling period. South African hybrids have a longer period to maturity than hybrids in the United States and are thus subject to blighting for a longer time during grain-fill. South African hybrids mature 120 to 150 days after planting, whilst commercial hybrids in the United States mature 30 to 35 days earlier. It is not surprising that Nutter (pers. comm., 1994)(1)8 concluded that the pathogen has a potential to cause higher yield losses in South Africa than in the United States.

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The recent rise in importance of GLS in South Africa and the extra costs involved in the management of the disease have placed financial pressure on the maize farmer to produce high quality maize crops at competitive prices. The most cost-effective and long-term solution to the problem is for maize-breeders to develop high-yielding hybrids that are resistant to the disease. Progress in breeding suitable resistant hybrids is, however, time-consuming, and this study is aimed at establishing economical alternative methods of control that can be used by producers as an interim solution. A thorough knowledge of the biology of the host and epidemiology of the fungal pathogen is necessary for the formulation of strategies for the control of the disease. This is because epidemiological differences exist among the host-pathogen interactions that enable methods of control to be developed. The development of a range of control measures requires studies on how the pathogen is influenced by the weather, hybrid susceptibility to disease, alternate hosts, the preceding crop, surrounding crops, cultural practices, level of inoculum and the use of fungicides. The infection process, including the spread of primary inoculum, spore germination, germ-tube growth and appressorium formation, penetration and internal growth in the host-lesion formation and the production of conidia, encompass the pathogen life-cycle. The interaction of the pathogen life-cycle on the biological processes of the maize-host (the disease-cycle) offers a basis for the formulation of epidemiologically justified control measures (Fig. 1).

The object of epidemiologically based control measures is to apply the most appropriate control measure at a time most likely to achieve the most efficient control at the most vulnerable stage of pathogen development. The ethograph of the GLS disease cycle illustrates these stages when control measures may be most efficiently applied (Fig. 1). These will be discussed in sequence of the stages in the disease cycle.

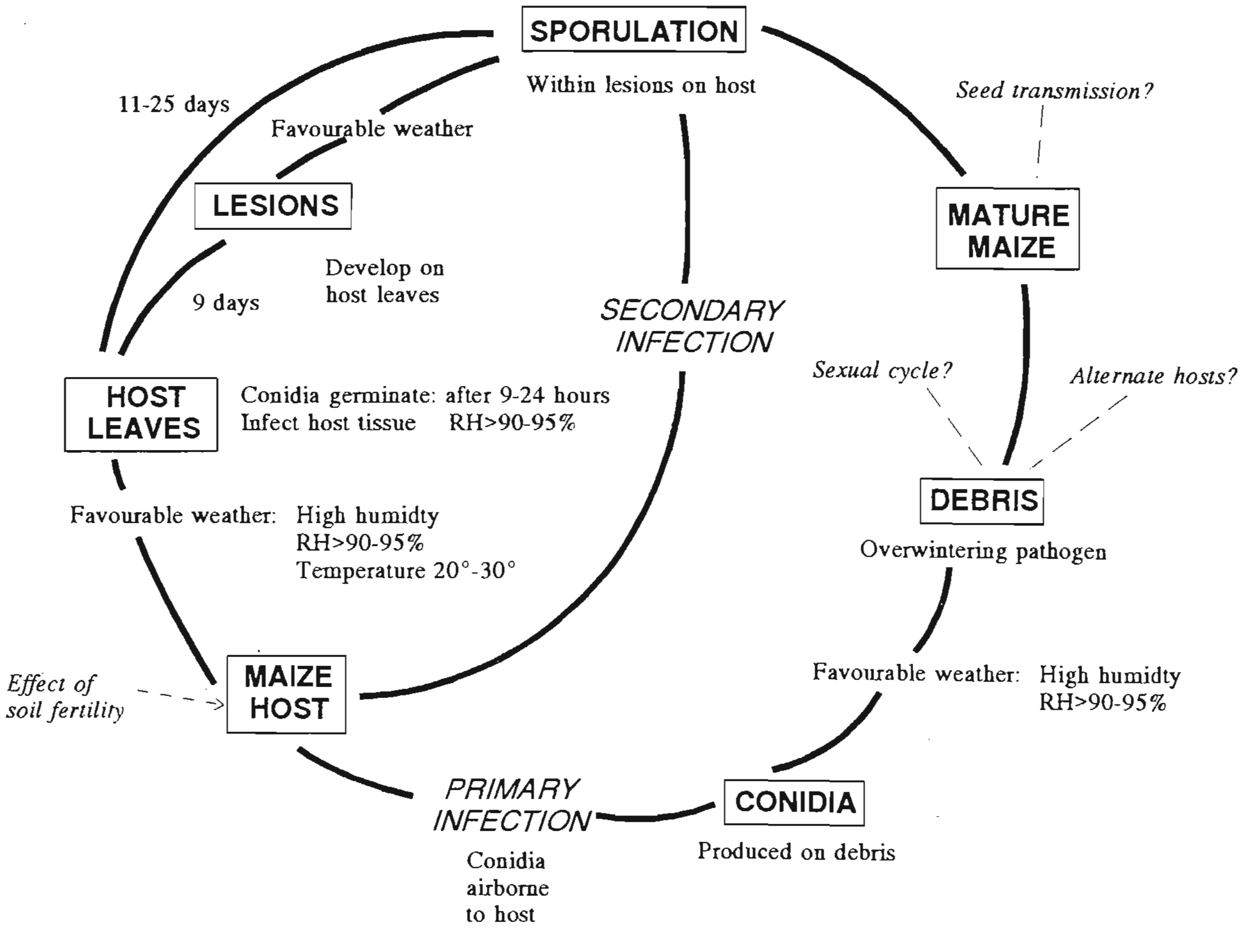

Figure 1 Disease cycle of maize grey leaf spot

8.1 DEBRIS

The pathogen can survive in the maize debris under adverse climatic conditions and remain capable of sporulation for up to 18 months in the form of stroma, provided the stubble does not decompose completely. Infected maize debris and stubble remaining on the soil surface are considered to be the main source of primary inoculum. Sporulation in the spring, after the following maize crop is planted, therefore provides the initial inoculum to infect the newly planted maize.

Management practices aimed at a reduction of initial inoculum are effective against monocyclic diseases, or diseases with long latent periods, especially diseases having a single host (Zadoks & Schein, 1979). *C. zeae-maydis* is a facultative saprophyte and is distinctly different from many other foliar pathogens of maize, in that it has a longer latent period. Although GLS is a polycyclic disease the fungus is only able to complete a few cycles of secondary inoculum spread in a single growing season, compared to many cycles completed by most other foliar pathogens. As maize is the only known host of the pathogen (Stromberg & Donahue, 1986), the severity of GLS in a region is dependent upon a large amount of initial inoculum from overwintering diseased maize debris on the soil surface. The amount of initial inoculum produced in the debris is an important determinant of the severity of GLS epidemics. Tillage practices, crop rotations, or the removal of the crop for silage aimed at the reduction of initial inoculum, are classical methods of control and have been demonstrated to be effective in managing GLS (Huff, Ayres & Hill, 1988; Latterell & Rossi, 1983). However, Perkins, Smith, Kinsey & Dowden (1995) inferred that ploughing and rotations are less effective in managing the disease in areas with high levels of inoculum, and where GLS is already established. This is largely because these regions have a large percentage of land in conservation tillage, and maize in conventionally tilled fields or maize fields following soyabeans may be damaged by GLS as a result of inoculum disseminated from adjacent fields where conservation tillage is used. Smith (1989) found more GLS in conventionally tilled treatments than in maize grown under stubble-tillage in four or five tillage trials. These results conflict with the findings of Payne, Duncan & Adkins (1987), who found more GLS in no-till plots. Smith (1989) suggested that the trials of Payne *et al.* (1987) were further removed from sources of inoculum than his trials and that practices aimed at the reduction of initial inoculum may only be effective in areas where external inoculum is minimal such as found in the trials of Payne *et al.* (1987). Where external inoculum is abundant and is responsible for early initial infection, control measures must be directed at the reduction in the rate of disease development (Smith, 1989). This is because of difficulty in managing inoculum from outside sources. Trials at Cedara have also indicated that conventional tillage is unlikely to have a major impact on the management of GLS, as the disease is well established in the region and there are abundant external sources of inoculum. Observations (results not shown) indicate that rotations with non-host crops are ineffective in managing GLS in areas where there is abundant external inoculum. However, rotations may have other beneficial effects such as improvement in soil structure, nutrient status and a reduction in soil pathogens detrimental to the growth of maize. These beneficial effects often result in increased maize yields, especially in seasons with low GLS disease. Further research on the effect of crop rotations is necessary to establish the precise effect of these practices on the development of GLS, especially in regions where the disease is less severe.

External sources of inoculum are not necessarily only from adjacent infected maize. Other sources of inoculum could result from commonly used production practices in South Africa, where maize crops are left standing in fields for grain to dry-down to approximately 13,5% moisture before harvesting. The dry-down often occurs in late winter, and stubble is only ploughed or tilled into the soil just prior to the planting of the next maize crop. During dry-down, frequent strong winds and 'dust-devils' in late winter often remove infected leaf-tissue and carry it long and short distances to areas adjacent to maize lands. This infected debris can frequently be found on grassed contours and headlands in and around maize lands, and can act as a source of inoculum to infect the newly planted maize the following spring.

The most effective control at the time of writing is the development and use of resistant hybrids in high-risk situations and more susceptible but higher yielding hybrids in lower-risk situations, in association with crop-rotations and conventional tillage.

8.2 CONIDIA AND INFECTION OF THE HOST

A notable feature of *C. zeae-maydis* is its ability to survive fluctuating weather conditions. Wind turbulence and rain-splash are important factors in dispersing conidia, especially as conidia are only loosely connected to conidiophores. Aerial spore counts are usually highest in the early afternoon (Payne & Waldron, 1983). Germination apparently must be initiated in a single period of wetness and is optimum at temperatures of 22 to 30°C (Rupe, Siegel & Hartman, 1982). Germlings (conidia with germ tubes), however, can survive for as long as 15 days under alternating conditions of RH, and have the ability to resume development when RH exceeds 95% (Thorson, 1989). Thorson's work indicated that appressorium formation requires 48 hours of humidity above 95% after germination at optimal temperatures. This period of high humidity does not have to be continuous, and if favourable weather conditions are interrupted, infection will be delayed until favourable humidity conditions return. The effect of these fluctuating humidity conditions might explain the variability in the latent period of 11 to 25 days (Thorson, 1989). This may be important in South Africa where there are fewer periods of high humidity in the early season than in the mid- and late-season, and may explain the reason for slower disease development in the early season. Recent disease prediction studies at Cedara support Thorson's postulations. Observations show that the pathogen requires a minimum period of high humidity at temperatures between 20 and 30°C for disease to develop. This period also does not have to be continuous and the pathogen can survive alternating periods with high humidity. Initial lesions show after 185 accumulated hours of RH above 95% at temperatures between 20 and 30°C, accumulated after spore germination. In seasons with more frequent periods of high humidity and temperatures above 20°C in the early season, the onset of disease is earlier, and the maize crop will require more frequent fungicide treatment for the management of the disease. These observations support the findings of Ringer & Grybauskas (1995), that early rains create favourable environmental conditions contributing to relatively high numbers of primary lesions that may provide sufficient inoculum to cause subsequent high levels of disease severity. Temperatures, especially lower temperatures, appear to play an important role in disease development. Field observations show that mean temperatures lower than 20°C, with RH above 95% resulted in fewer primary lesions and low final disease severity.

8.3 MAIZE HOST

Hybrid resistance is the most effective and cost-efficient way of managing GLS and is aimed at reducing the rate of disease development (Coates & White, 1995; Lipps & Pratt, 1989). No commercial hybrids have been identified in South Africa that are resistant to the pathogen (Ward & Nowell, 1997), but Gevers and Lake (1994) have identified a major gene for resistance to the pathogen in inbred lines. This gene could be easily and rapidly incorporated into elite breeding material in a simple back-cross programme (Gevers & Lake, 1994). Gevers[1] (pers. comm.) believes this could offer a highly rewarding solution to the problem of GLS in South Africa. However, a single-gene mutation in the pathogen could just as easily and rapidly overcome resistance based on a single dominant gene. For this reason, rate-reducing polygenic resistance is usually preferred (Latterell & Rossi, 1983). Breeding for polygenic resistance is additive and was not very complex (Thompson, Bergquist, Payne, Bowen & Goodman, 1987). This is because the resistance is regulated by a small number of genes, but each of which adds small increments of resistance to the hybrid (Ayres, Johnson & Hill,

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[1] Gevers, H.O., 10 Spearman Drive, Hayfields, Pietermaritzburg, 3201, South Africa

1985). In order to achieve a high level of resistance, the hybrid would require more genes for resistance, whilst fewer genes would be necessary for lower levels of resistance. A breeding programme which incorporates several genes for high level resistance in a hybrid would take several years. This is the likely explanation for Anderson (1995) suggesting that the level of resistance required in any breeding programme is a critical question. If too high a level of resistance is required, breeders may be forced to sacrifice other goals, such as yield, in the breeding programme, as it would take several seasons to develop the level of resistance required. On the other hand, the development of lower levels of resistance, which is less time-consuming, may ensure hybrids with higher yields, although these may be at greater risk under GLS. Breeding for polygenic resistance is therefore time-consuming, and the selection of high-yielding hybrids less-susceptible to GLS should remain an important priority in any research programme until the desired level of polygenic resistance can be introduced. The development of marker-assisted breeding programmes in the future may hasten the development of acceptable GLS-resistant hybrids. Research on cultivar susceptibility to *C. zeae-maydis* at Cedara, under conventional and stubble tillage, has shown that linear regression of relative yield against relative disease severity enables the identification of high-yielding maize hybrids less susceptible to the pathogen or tolerant of the disease. Only less susceptible hybrids that have the ability to produce high grain yields should be selected in areas where GLS is present. Hybrids more susceptible to disease should be avoided. As a result of this work, these high-yielding, less-susceptible hybrids have largely replaced the high-yielding but highly susceptible hybrids that were previously widely grown in

KwaZulu-Natal (Ward & Nowell, 1997).

Fungicides have been shown to reduce disease severity in hybrids. Linear regression of gain in yield (response) due to fungicide treatment against disease severity enables the identification of hybrids with optimal response to fungicides. Hybrids less-susceptible to the pathogen are at lower risk to serious yield loss and are likely to require fewer fungicide treatments than more-susceptible hybrids. These less-susceptible hybrids, that are higher yielding, are recommended in areas where GLS is a problem and where fungicides are usually used. It is suggested that these hybrids have some genes for resistance, which is encouraging, as the genes from the inbred parent-lines can be exploited to find the long-term solution to the problem.

The ability to predict hybrid yield responses to fungicide treatment under varying levels of disease can assist the decision whether or not to use fungicides. This is useful in dry seasons with low disease levels when such decisions are most difficult. The regression model presented in this thesis makes this possible, as it is capable of predicting yield responses under varying levels of disease. Under low levels of disease, the lower than predicted yield response of some less-susceptible hybrids to fungicide treatment may not justify spraying. Spraying is justified only when the predicted yield response to fungicide treatment provides a higher financial return than the cost of fungicide treatment.

Fungicides reduce the rate of disease development in KwaZulu-Natal and, because of the studies presented in this thesis, fungicides are now widely used by commercial farmers. The correct time of application is important for effective control, while the number of spray treatments is determined by the growth stage of maize when the disease first develops. The financial analysis of fungicide treatment, however, indicates that the treatment resulting in the highest yield is not always the most economical treatment. Farmers should therefore consider the financial implications, especially the expected added income compared with the added costs for fungicide treatment. The most appropriate treatment choice will depend on the individual farmer's risk-aversion preferences.

Systemic fungicides have a 'curative' action and can be applied after disease is observed, whereas contact fungicides must be applied in a preventative spray programme before disease develops. Only systemic fungicides have been registered for the control of GLS in South Africa. These fungicides interfere with a specific biochemical process of the pathogen to effect control, and are known as "site-specific" fungicides. Fungal pathogens are able to circumvent, and have circumvented, the activity of some site-specific fungicides, thus developing resistance. In some cases resistance has developed rapidly, as has been reported with fungicides of the benzimidazole group (Smith, 1988). The development of resistance is more common with fungi having polycyclic rather than monocyclic life-cycles. As *C. zeae-maydis* is a facultative polycyclic pathogen and because benomyl (a benzimidazole) was the first fungicide registered for GLS control, there is always the threat of strains of *C. zeae-maydis* developing resistance to benomyl. Trials evaluating fungicides with different modes of action were successfully conducted at Cedara and only products containing mixtures of triazole and benzimidazole fungicides are now registered in South Africa. These mixtures, with multi-site action, provide longer and more effective control of GLS and result in higher grain yields than site-specific fungicides. The benzimidazoles (*e.g.* benomyl) appear to have little effect on plant growth (Smith, 1989). Triazoles, on the other hand, have growth regulating properties (Lonsdale & Kotzé, 1993), and this may account for higher grain yields obtained from the fungicides of this group. The higher yields, however, were more likely to have been the result of the possibly broader control spectrum of the mixture, permitting concomitant control of other diseases such as rust (*Puccinia sorghi* Schw.), eyespot (*Aureobasidium zeae* Narita & Y. Hiratsuka) and, possibly, soil pathogens.

The effectiveness of fungicide treatments is influenced by the correct timing of spray applications and the number of spray treatments applied. Correct timing of treatments is based on infection levels of the pathogen, while the number of applications is based on both growth stage of the maize plants when treatments are initiated as well as the prevailing weather conditions. Research at Cedara demonstrated that the most appropriate time to commence spraying was during the exponential phase of the disease epidemic, when disease severity was 1 to 2% and symptoms were present on the basal five-leaves of the maize plant. Spraying earlier in the exponential phase, at lower disease severity, resulted in a shortening of the effective period of control. Treatment later, during the logistic phase of the epidemic, when secondary inoculum from the primary lesions resulted in a faster growth of disease epidemic, also resulted in a shortening of the effective period of control.

The number of spray applications is directed at delaying disease development. The objective of the spray-programme is to maintain healthy photosynthetic leaf tissue on the plant, above ear-height, until the crop is physiologically mature. The number of spray treatments will therefore depend on the growth stage of the maize plant when spray treatments are initiated. This will also determine the period until physiological maturity, during which control is required. For example, if the initial spray treatments are initiated 90 days after planting, and physiological maturity is estimated to be 145 days after planting, the crop requires protection for a period of 55 days. Since fungicides provide between 29 and 32 days of control, more than one spray treatment will be necessary for effective control of GLS.

The aerial application of fungicides commenced on a limited scale in the 1991/92 season. The swath widths used and spray volumes applied were based on limited trial work conducted by the author, and have not been presented in this thesis. Good control of the disease was achieved, and this was reflected in improved maize yields. However, strips of maize in which disease was not controlled were observed in treated fields, and this striping effect was considered to be the result of poor calibration of the spray aircraft. Fungicide efficiency can be improved by the proper calibration of spray aircraft, the addition of spray adjuvants, and possibly by the use of more modern spray equipment fitted to the aircraft. The effects of anti-evaporants and anti-drift agents added to the fungicide spray mixtures have not been fully investigated. The application of fungicide spray solutions using micronair spray equipment may also lead to improved disease control by better spray coverage of plants. Micronair sprays may lead to lower application costs by applying lower spray volumes. Further research on these aspects is necessary as their use may lead to improved fungicide recovery and reduced costs of application.

A recent development in the application of fungicides for the control of GLS has been the introduction of high-clearance tractor sprayers. These sprayers appear to apply the fungicide solutions efficiently, but they may also damage maize plants from tractor wheels during application, especially in areas where maize rows are unevenly spaced. More careful planting with accurate row spacings, suitable for tractor sprayers, would lead to improved fungicide application. A comparison of the cost-effectiveness of ground and aerial application may determine the most effective method of application. A preference by farmers for either aerial or ground spraying could lead to the development of financially rewarding spray contract business.

A hand-held overhead sprayboom attached to a back-pack sprayer has been developed and evaluated at Cedara. This has provided effective control of GLS and is a method of application ideal for treatment on small areas of maize and for small-scale farmers.

Spray prediction models have been developed successfully and used to determine the correct timing of fungicide treatments for the control of many diseases of different crops. Many of these have resulted in a reduction of fungicide use, a lowering of input costs and increased returns to farmers without affecting disease control efficiency. Until hybrids resistant to GLS become commercially available there will be a need for such a spray advisory for the control of GLS in South Africa. The development of a computerised spray advisory, based on the growth responses of *C. zeae-maydis* to specific environmental conditions, would assist farmers and advisors greatly in the proper use of fungicides. A spray advisory is currently being developed and evaluated at Cedara, but for greater precision more information on the infection process of the pathogen under South African conditions is necessary.

Small-scale farmers prefer open-pollinated cultivars to hybrids. These are popular, as seed can be kept from the harvested crop to plant in the following season. Seed is therefore cheaper than hybrid maize, and small-scale farmers prefer the taste of open-pollinated to that of hybrid maize. Limited research has been conducted on the susceptibility of open-pollinated maize to GLS. Results from the present author's work (not presented in this thesis) indicate that lower yielding open-pollinated maize is also susceptible to GLS. While GLS has been observed in small-scale farming areas, little is known of its potential to cause economic hardship and food-shortage amongst the rural community. It is therefore suggested that surveys be conducted to establish the disease potential in rural farming areas. More detailed research is required to identify higher-yielding open-pollinated cultivars that are less susceptible to GLS, as small-scale farmers either currently cannot afford the extra costs of chemical treatment, or are insufficiently practised in the application of fungicides for the control of disease. These aspects are becoming increasingly important in South Africa today, as emphasis is being given to self-sufficiency in the rural areas.

Little work has been undertaken on the effect of soil nutrients on the development of GLS on maize. Smith (1989) found that the application of nitrogen had a significant positive impact on the development of GLS, whilst potassium had surprisingly little effect. Phosphorus, similarly, had little effect. It is possible that relatively high soil potassium and phosphorus levels may have affected GLS responses in Smith's trials. Observations (results not shown) at Cedara of plots deficient in potassium and maize deficient in nitrogen were less infected by *C. zeae-maydis*. This is contrary to the effect of potassium on many other foliar pathogens. As much maize in rural areas is grown on nutrient-deficient soils, and because of official policies that encourage the use of fertilizers to improve yields in these areas, these practices may lead to increased risks from GLS and may require costly control measures. The effects of fertility on susceptibility of maize to GLS is a field that should be researched further.

A feature of GLS is the suddenness with which it has risen to become a major potential threat to the maize industry, especially in KwaZulu-Natal. The disease was first observed in 1988 and since the 1990/91 season has caused significant financial losses to farmers (Ward & Nowell, 1994). The origin of the disease in South Africa has been the subject of much speculation. Gevers (pers. comm.), a notable maize breeder in South Africa, is of the opinion that *C. zeae-maydis* has been present in South Africa for many years. He has suggested that a more favourable climate and an increase in reduced tillage practices in recent years has led to the increase in GLS. He believes that it may also be due to genetic vulnerability in locally used breeding material. This genetic vulnerability is a likely cause of the increased prevalence of GLS, rather than reduced tillage practices. Reduced tillage practices have, in fact, declined since the mid-1980s, following ear-rot epidemics when farmers sought solutions by reverting to conventional tillage (Gevers & Lake, 1994). There have been other suggestions that the disease may have entered South Africa with imported breeding material. However, as there is no record of the disease being seed-borne, this is unlikely. A more likely explanation for the occurrence of GLS is the presence of infected debris in imported grain from the United States, following severe droughts in South Africa in the 1980s. Infected debris may have become wind-borne during off-loading operations, to infect nearby planted maize. This possibility has led to recent requests for tightening of import control measures on maize from abroad to prevent the possible entry of other pathogens.

McGee (1988) lists barnyardgrass (*Echinochloa* sp.) and *Sorghum* spp. as alternate hosts for GLS of maize. These are alternate hosts for *C. sorghii* (Frederiksen, 1986), and it is possible that McGee (1988) implied these to be alternate hosts for *C. sorghii*, as he listed *C. sorghii* incorrectly as an additional causal pathogen of GLS disease of maize. If *C. sorghii* has other graminaceous hosts, it may be possible for *C. zeae-maydis* to have alternate hosts also, but these have not been identified. Should there be alternate hosts for *C. zeae-maydis* which are capable of providing sources of inoculum, then management practices such as tillage and crop rotations aimed at the reduction of initial inoculum may be less effective. This would have serious consequences in areas with lower maize yield potentials and possibly lower inoculum levels. Tillage practices and crop rotations in these areas would offer financially acceptable control measures, while the extra costs, should fungicide control be necessary, may reduce the financial return and render the production of maize in these areas uneconomical. Searching for alternate hosts of *C. zeae-maydis* should therefore be thorough and the effectiveness of crop rotations and tillage practices in managing GLS in these lower-potential maize-growing areas should be thoroughly researched.

Though rated as being of little epidemiological importance, Latterell & Rossi (1983) observed the perfect stage of *C. zeae-maydis* as an unnamed species of

Mycosphaerella in overwintering debris. The occurrence was rare, indicating that it was not a source of inoculum in the spring in the United States, and that it is not the imperfect stage (*C. zeae-maydis*) that is responsible for the spread of the disease. Usually the importance of a sexual stage is considered to be in the spread of the pathogen over long distances and in diverse genetic recombinations. The implications of a sexual stage in the disease cycle could be serious in South Africa as it would add genetic variability to the pathogen during recombination in the sexual process. Such increased variability would increase the adaptability of the fungus to new environments, thereby enabling it to establish under a wider range of environmental conditions. The variability would also result in more rapid development of resistance to fungicides, or virulence to host-resistance, especially if the resistance was due to vertical resistance. The occurrence of the perfect stage would increase the importance of control measures of the fungus in infected debris. Although the occurrence of a perfect state in the United States may be rare, it may be more common in South Africa, where the winter weather is less harsh than in the United States, and this aspect should be the subject of further investigation.

Management practices aimed at the control of GLS have been based on the epidemiology of the fungal pathogen. An integrated approach using tillage practices, crop rotations and hybrids less susceptible to the pathogen, and the judicious use of fungicides, are likely to be the main control measures, but in the long term the cornerstone of the integrated approach will be the development of resistant hybrids. High yielding, well adapted hybrids, less susceptible to the pathogen, should be planted in all areas where GLS is present. Crop rotations are more likely to be successful if implemented on a regional scale, using less-susceptible hybrids to GLS, since there is always a danger that wind may blow in conidia from adjacent infected maize, if large areas are not planted to rotations. Tillage practices are more likely to be effective in areas where the disease is not already established and where inoculum levels are relatively low. In areas where GLS is well established and inoculum levels are high, fungicides are most likely to provide effective control.

8.4 FUTURE RESEARCH NEEDS

8.4.1 Epidemiology studies in Africa

An understanding of the epidemiology of *C. zeae-maydis* is fundamental to the development of strategies for its control. The only studies on the epidemiology of the pathogen have been conducted in the U.S., and it is possible that differences in the pathogen development between continents may exist. It is important that such studies be conducted in Africa, where the disease is spreading to countries throughout the continent. This would enable the formulation of local strategies for control of the disease in Africa. In particular, it is suggested that studies should be conducted on the stages of pathogen development on hybrids of different susceptibility to disease under varying environmental conditions. Data generated from such studies may allow for the formulation of appropriate control measures at times most likely to achieve control at the most vulnerable stage of pathogen development.

8.4.2 Reduction of initial inoculum

Further study of crop rotations and tillage practices aimed at the reduction of initial inoculum on diseased debris as a management strategy to manage GLS in areas where GLS is present but which are at lower-risk from the disease, should enjoy a high priority in any future research programme.

8.4.3 Reduction in the rate of disease development

8.4.3.1 *Breeding for resistance*

Recent advances in the use of genetic markers such as DNA restriction fragment polymorphism (RFLP) to identify genes that control quantitative traits or quantitative trait loci (QTL's) are recommended. Effective control of GLS can be obtained by incorporating resistance in high yielding hybrids adapted to South African conditions. This will require the use of biotechnological techniques such as RFLP directed breeding programmes to hasten the success in identifying genes and QTL's in elite breeding programmes.

8.4.3.2 *Hybrid trials*

Evaluation of hybrids for susceptibility to GLS should be ongoing. Such studies should also investigate the effect of blight stress on other pathogens namely stalk-rotting pathogens as hybrids susceptible to GLS are more likely to suffer greater yield loss under GLS pressure.

8.4.3.3 *Fungicide evaluation*

Fungicides belonging to the triazole and benzimidazole chemical groups are registered and are effective in the control of GLS. However, as part of a resistance management strategy, it is important to identify fungicides of different chemical groups, which act on different sites on the biochemical processes in the pathogen. Such research would be a precaution against the development of resistance by the pathogen against currently registered products.

8.4.4 **General**

8.4.4.1 *Effect of nutrition on GLS*

Little research has been conducted on the effect of soil nutrients on the development of GLS, but observations (pers. obs.) indicate that disease severity increases with increased levels of nitrogen and potassium. This has implications in small-scale farming areas where maize often grown on nutrient-deficient soils and where official policies encourage the use of fertilizers to improve yields. It is possible that such policies may lead to increased risks from disease by increasing the nutrient content of plants.

8.4.4.2 *Development of spray prediction models*

The development of spray advisories for early-warning of disease in maize crops would assist farmers and advisers in spray decisions. This would assist in the determination of correct timing of fungicide treatments.

8.4.4.3 *Aerial application of fungicides*

Effective control is achieved using aerial spray techniques. However, improvements to aerial spraying techniques may lead to improved GLS control. The development and use of micronair spraying equipment may improve spray coverage of maize plants, improved control of disease and reduced costs of application.

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