Studies on Ear Rot and Grey Leaf Spot of Maize in South Africa

by

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(B.Sc.Agric.)

Submitted in partial fulfilment
of the requirements for the degree of

DOCTOR OF PHILOSOPHY IN AGRICULTURE

in the
Department of Microbiology and Plant Pathology
Faculty of Agriculture
University of Natal
Pietermaritzburg
South Africa

May 1997

ABSTRACT

In recent years there have been economically important epidemics of both *Stenocarpella* ear rot and grey leaf spot (GLS) in South Africa. These epidemics have adversely affected the grain yield and quality of the maize harvested. Maize researchers and breeders have had to re-assess the importance of maize disease in South Africa and make the necessary adjustments to their programmes. Literature reviews were undertaken on both *Stenocarpella* ear rot and GLS to provide the necessary background of technical information to conduct research under local conditions into these disease problems, and to assist in interpretation of results of experiments.

A novel method of inoculating milled *Stenocarpella* infected ears into the whorls of maize plants (about 2 weeks before 50% anthesis) was developed to provide consistent inoculum pressure and increased ear rot. This inoculation method was practical, efficient, reliable, consistent and cheap to implement. Commercial organisations could use this inoculation method to inoculate a large number of plants per day, allowing for improved screening of breeding material and hybrids.

Ear rot assessment methods, and researchers' ability to assess ear rot, were tested under South African conditions. The accuracy of the different methods tested varied considerably, particularly when there was a high level of ear rot that could not be seen without shelling the grain. Each method could be used in a maize breeding programme, depending upon the desired levels of accuracy and time taken using the given method. Researcher's ability to assess ear rot varied considerably and accuracy was correlated with the number of years experience in maize research. Grain colour affected the researcher's ability to accurately assess ear rot severity. Yellow-grained maize was more difficult to assess for ear rot than white-grained maize.

Hybrid response to *Stenocarpella* ear rot infection was difficult to interpret owing to a significant interaction with the environment. Hybrid ear rot response was non-linear in nature. Normal methods of presenting disease data and classifying hybrids in resistance response categories were not successful. Non-linear regression analysis has to be used to do this. However, it is important that ear rot data be presented in a way that farmers can utilise the information. Pre-flower stress predisposes maize hybrids to ear rot infection. Hybrids that

normally exhibited good levels of resistance to *Stenocarpella* ear rot may become severely colonised if drought stress occurs in the four weeks prior to flowering. This environmental interaction makes ear rot resistance breeding and the interpretation of results difficult.

The incidence of maize ear rot was widely considered to increase with increased plant density. Experiments over three seasons in South Africa have shown that is not true under certain environmental conditions. In specific hybrids, plant densities of less than 50 000 plants ha^{-1} exhibited a higher incidence and severity of ear rot than plant densities greater than 50 000 plants ha^{-1}. The hybrids that usually responded in this manner were more susceptible to ear rot than the other hybrids. Generally, ear rot increased with increased plant densities over 50 000 plants ha^{-1}. The mechanisms and reasons for this could not be determined.

Fungicide trials and regression analysis of hybrid yield trials over a two years period, at two locations in KwaZulu-Natal, showed that grain yield losses due to GLS infection were at least 13%. Severity of GLS was consistently higher at Cedara than at Greytown. Economic losses at Cedara ranged from R1 919 - R2 278 ha^{-1} and at Greytown from R1 554 - R1 726 ha^{-1}. Predicted hybrid losses ranged from R836 - R2 621 ha^{-1} (13% - 37%), depending upon the level of inherent GLS resistance.

Hybrid response to *Cercospora zeae-maydis* infection was linear in nature and hybrids could be categorised into response categories. Large differences in GLS resistance could be found between commercial hybrids. However, the current levels of GLS resistance in hybrids does not eliminate yield loss under high GLS inoculum levels, and fungicide application was economically justified on most hybrids. Newly released hybrids show increased levels of GLS resistance.

The application of systemic fungicides to GLS-susceptible maize was highly effective in controlling GLS and increasing yield substantially. The most effective fungicides belonged to the triazole and benzimidazole group of fungicides. Protectant fungicides were not as effective as systemic fungicides. Copper-based fungicides were phytotoxic to maize in two seasons and at both locations. Fungicide mixtures of the two groups active against GLS are being used on commercially. The effectiveness of fungicides did not vary over location or hybrids, but was influenced by inoculum pressure.

Effective control strategies have been implemented to control both *Stenocarpella* ear rot and GLS in South Africa. Crop rotation, the selection of the more ear rot and GLS-resistant hybrids, and the judicious use of fungicides has reduced the levels of both diseases to manageable levels. An integrated control strategy is needed to control these diseases and efforts are being made to educate farmers to this effect. Maize pathological research now enjoys a greater emphasis than it did in the early-1980s.

DECLARATION

I, David Carlisle Nowell, declare that this dissertation, except where otherwise indicated, is my own original research. It has not been submitted in part or as a whole, for a degree at any other university.

David C. Nowell

FOREWORD

This thesis has evolved over a number of years and has concentrated on the most important maize diseases during the period in which the trials were undertaken. The thesis has been divided into four sections: general introduction, ear rot research, grey leaf spot (GLS) research and a general discussion. In the case of the research sections, each section is started with a literature review for the particular disease or phase of the disease. Each chapter has been written as a discrete paper (with the intention of publishing these data in the near future) and this has resulted in some duplication in the introductions and references between chapters. The advantage is that each chapter can be read independently of each other.

Local objectives and constraints shaped the structure and shape of the thesis. When maize pathology research was initiated at Greytown in December 1981, ear rot had not yet reached epidemic proportions but was considered a high priority. As the ear rot epidemics developed, so the priorities and objectives of the research were changed, often in the middle of a growing season. Some of the data on plant densities and ear rot was collected on trials intended for yield purposes only and were established by other members of Pannar Seed (Pty) Ltd and Pannar Research Services (Pty) Ltd. The collection of data and data analysis was undertaken independently from these people.

The research on GLS developed after the initial identification of the pathogen in the Greytown area, and then the subsequent rapid spread and increased severity of the pathogen. The start of the GLS epidemics coincided with the end of the ear rot epidemics. The GLS research was conducted in association with J.M.J. Ward (Cedara Agricultural Development Institute) at Cedara, near Pietermaritzburg because of the diversity and magnitude of research undertaken, and the urgent need for comprehensive solutions to the problem. The two research programmes of Nowell and Ward were designed to run in parallel, to optimise effort, and to ensure that the results were applicable to the region as a whole. All data from Cedara resulted from trials conducted by Ward and have been incorporated into this thesis to add depth and significance to the findings for KwaZulu-Natal as a whole. The collaboration on these trials was from the planning stage to the analysis and writing up of these trials.

The research in this thesis was based on the maize industry's need for quick, practical and cost effective solutions to the predominant maize disease in the 1982 - 1996 period. All research

was undertaken within the Research Department of Pannar Seed (Pty) Ltd on the Greytown research farm in KwaZulu-Natal. This region is a 'hot spot' for maize diseases due to the favourable climate for disease, which often become epidemic in this region before they are problematic in other parts on the maize production region.

Research within Pannar Seed (Pty) Ltd allowed for a rapid response to solve disease problems, flexibility in research projects and the rapid feedback of practical solutions to extension, marketing and production personnel within the company. The research results and solutions to disease problems were immediately passed on to the farmers and farming community in general. The research reported in this thesis is less than 10% of the research actually conducted during this period, and only research on the two most economically important diseases to the maize industry is reported.

Some of the information in this thesis has already been partially published or has been accepted for publication:

Nowell, D.C. 1989. Some aspects of ear rot data collection and presentation. Pgs 68-72 in: Proc. 8th. S.Afr. Maize Breeding Symp., Potchefstroom 1988, J.G. du Plessis (Ed.), Tech. Comm. No. 222, Dept. Agric. and Water Supply, Pretoria, RSA.

Nowell, D.C. 1989. Maize ear rot data presentation. (Abstract). Phytophylactica 21:103.

Nowell, D.C. 1992. Modified breeding strategies for ear rot resistance in maize under reduced tillage. Pgs 53-59 in: Proc. 9th. S.Afr. Maize Breeding Symposium, Cedara 1990, H.O. Gevers (Ed.), Tech. Comm. No. 232, Dept. Agric. And Water Supply, Pretoria, RSA.

Nowell, D.C. 1995. Breeding, screening and evaluation strategies for maize ear rot resistance. Pgs 154-146 in: Proc. 4th Eastern and Southern Africa Regional Maize Conference, 29 March - 1 April, 1994, CIMMYT, Mexico.

Ward, J.M.J. and Nowell, D.C. 1997. Epidemiology and management of grey leaf spot disease, a new disease of maize in South Africa. In: Proc. 11th S. Afr. Maize Breeding Symp., Cedara 1994, H.O. Gevers (Ed.), Grain Crops Research Institute, Dept. Agric., Pretoria, RSA. (In Press).

Ward, J.M.J., Laing, M.D. and Nowell, D.C. 1997. Chemical control of maize grey leaf spot. Crop Prot.. (Accepted for publication but due in mid-1997).

Ward, J.M.J., Birch, E.B., and Nowell, D.C. 1993. Grey leaf spot on maize. Coordinated extension: Maize in Natal, Cedara Agricultural Development Institute, Pietermaritzburg, RSA. pp. 10.

A considerable amount of detail has now been added to the above papers and the updated research results will be published in peer reviewed journals.

The numbering method used for the tables and figures in the different chapters is the number of the chapter, a full stop, followed by the sequential number of the table or figure in that chapter. The tables and figures in the General Introduction and General Discussion are sequential over both chapters and reflect the number of the table or figure only.

ACKNOWLEDGMENTS

To my wife Leanne and children, Megan, Brandon and Tyrone, a big thank you for their continued love, support and endurance over the past years. Without their sacrifices and understanding it would not have been possible to have even attempted writing this dissertation. I wish to express my appreciation and thanks to my parents and family for their support and encouragement.

Without employment by Pannar Research Services (Pty) Ltd and their continued support and guidance over the years, this thesis would not have been possible. In particular, I would like to thank Ian Jarvie, Heinz Kaiser and Tony Farwell for their input and guidance into the pathology programme overs the years. My thanks to Ash Babooram for his technical assistance and my colleagues who have provided advice and comments.

My sincere appreciation to Professor F.H.J. Rijkenberg, my supervisor, for finding the time to assist in producing this dissertation.

I am extremely grateful to Mark Laing, my co-supervisor, for all his time, assistance, advice, understanding and support during the writing of this dissertation.

My thanks to Pat Caldwell for finding the time to edit some chapters in this dissertation so efficiently.

Sincere appreciation and thanks go to Margie Whitwell for her guidance and assistance in statistical matters over the past two years.

My appreciation and thanks to the Agricultural Research Council for allowing the use of data from some of their Phase II maize trials. My sincere appreciation to Bradley Flett for his willingness to discuss ideas, cooperate and improve our understanding of maize ear rot and associated diseases.

My sincere thanks, appreciation and gratitude to Julian Ward for his openness and willingness to cooperate, share information and for many frank discussions regarding the Grey leaf spot (GLS) research. Without this we would not have been able to accomplish the tremendous understanding of GLS of maize that has been achieved in the RSA. The results of this collaboration have been invaluable to researchers and farmers alike.

CONTENTS

ABSTRACT i

DECLARATION iv

FOREWORD v

ACKNOWLEDGMENTS viii

CONTENTS ix

GENERAL INTRODUCTION 1
LITERATURE CITED 6

SECTION I - EAR ROT

CHAPTER 1

A Review of *Stenocarpella* Ear Rot of Maize 11
ABSTRACT 11
1.1 INTRODUCTION 11
1.2 CROP RESIDUE AND TILLAGE PRACTICES 17
1.3 CROP ROTATION AND SANITATION 18
1.4 HOSTS AND DISTRIBUTION 19
1.5 INFECTION OF THE HOST, SYMPTOMS AND DISEASE DEVELOPMENT 20
1.6 HOST RESISTANCE 24
1.7 OTHER FACTORS 31
1.8 LITERATURE CITED 35

CHAPTER 2

Reaction of Maize Hybrids to a Non-invasive *Stenocarpella* Ear Rot Inoculation Technique in KwaZulu-Natal 40
ABSTRACT 40
2.1 INTRODUCTION 40
2.2 MATERIALS AND METHODS 41
2.3 RESULTS 43
2.4 DISCUSSION 67
2.5 LITERATURE CITED 70

CHAPTER 3

Assessment of Maize Hybrids for Ear Rot Infection in South Africa 71
ABSTRACT 71
3.1 INTRODUCTION 71
3.2 MATERIAL AND METHODS 72
3.3 RESULTS 75

3.4 Discussion 81
3.5 Literature Cited 84

CHAPTER 4

Responses of Maize Hybrids to Ear Rot Infection in South Africa 85
Abstract 85
4.1 Introduction 85
4.2 Material and Methods 86
4.3 Results 88
4.4 Discussion 97
4.5 Literature Cited 100

CHAPTER 5

Ear Rot Incidence in Maize Hybrids at Various Plant Densities In KwaZulu-Natal, South Africa 102
Abstract 102
5.1 Introduction 102
5.2 Materials and Methods 103
5.3 Results 105
5.4 Discussion 118
5.5 Literature Cited 121

CHAPTER 6

Breeding Strategies for Ear Rot Resistance in Maize Under Conservation Tillage . 122
Abstract 122
6.1 Introduction 122
6.2 Conservation Tillage 123
6.3 Artificial Epidemics 125
6.4 Resistance 127
6.5 Disease Assessment 132
6.7 Literature Cited 133

SECTION II - GREY LEAF SPOT

CHAPTER 7

A Review of Grey Leaf Spot of Maize 137
Abstract 137
7.1 Introduction 137
7.2 Crop Residue and Tillage Practices 141
7.3 Crop Rotation and Sanitation 142
7.4 Hosts and Distribution 143
7.5 Infection of the Host and Disease Development 144
7.6 Symptoms and Toxin Activity 146
7.7 Host Resistance 148

7.8 CHEMICAL CONTROL 153
7.9 OTHER FACTORS 159
7.10 YIELD LOSS 161
7.11 LITERATURE CITED 161

CHAPTER 8

Economic Losses as a Result of Grey Leaf Spot on Maize in KwaZulu-Natal, South Africa 169
ABSTRACT 169
8.1 INTRODUCTION 170
8.2 MATERIALS AND METHODS 171
8.3 RESULTS 174
8.4 DISCUSSION 185
8.5 LITERATURE CITED 190

CHAPTER 9

The Use of Fungicides to Control Grey Leaf Spot on Maize in South Africa 192
ABSTRACT 192
9.1 INTRODUCTION 193
9.2 MATERIALS AND METHODS 194
9.3 RESULTS 199
9.3 DISCUSSION 211
9.4 LITERATURE CITED 217

CHAPTER 10

Maize Hybrid Response to Grey Leaf Spot Under Two Tillage Systems In South Africa 221
ABSTRACT 221
10.1 INTRODUCTION 221
10.2 MATERIALS AND METHODS 222
10.3 RESULTS 226
10.4 DISCUSSION 238
10.5 LITERATURE CITED 240

SECTION III - GENERAL DISCUSSION

GENERAL DISCUSSION 243
1 EAR ROT 243
2 GREY LEAF SPOT 267
3 MANAGEMENT STRATEGIES 295
4 LITERATURE CITED 301

GENERAL INTRODUCTION

The Republic of South Africa (RSA) is a country with a wide range of land types, from desert to sub-tropical forest, that allows for a wide range of agricultural uses. Approximately 68% of the land is natural grass, while only 11% of the country is cultivated. White grained maize is the staple diet of most South Africans and a considerable amount of yellow maize is produced for animal consumption. The area planted to maize varies considerably but is usually between 3.5 - 4 million hectares per annum. This is 25 - 29% of the arable land usage or approximately 3% of the total area in South Africa. Maize is the most important cereal crop (Anonymous, 1989). From Table 1 it can be seen that the total consumption of maize in South Africa is approximately 6 million tons.

Table 1: The breakdown of maize consumption (kilotons) in South Africa in the 1985/86 to 1994/95 marketing seasons (Anonymous, 1995)

Marketing season	Human consumption	Animal consumption	Industrial consumption	Losses	Total
1985/86	2594	2722	163	-	5479
1986/87	2508	2556	136	6	5206
1987/88	2693	2523	161	6	5371
1988/89	2631	2766	160	6	5563
1989/90	2560	3462	212	8	6242
1990/91	2818	3557	226	-	6601
1991/92[1]	2877	3775	219	-	6871
1992/93	2780	3639	217	11	6647
1993/94	2750	3484	237	-	6471
1994/95	2583	3517	268	8	6376

[1] During the 1991/92 marketing season 89kt of imported wheat grain was used as stock feed in lieu of yellow maize.

Table 2: The area planted, mean grain yield and total production in specific countries that production significant quantities of maize grain for the years 1990 to 1995 (Anonymous, 1995)

	Area ('000 ha)				Yield (t/ha)				Production (kt)			
Country	1991	1992	1993	1994	1991	1992	1993	1994	1991	1992	1993	1994
USA	27861	29203	25492	29278	6.82	8.25	6.32	8.69	189885	240846	161146	254274
China	21649	21118	20769	21161	4.58	4.53	4.96	4.93	99094	95722	103046	104350
Brazil	13064	13364	11868	13725	1.81	2.28	2.53	2.34	23624	30506	30065	32136
Mexico	6947	7219	7397	7000	2.05	2.34	2.43	2.37	14253	16929	17964	16600
Argentina	1918	2367	2503	2422	4.05	4.52	4.36	4.31	7768	10699	10901	10439
South Africa	3487	3663	3904	2952	0.85	2.48	3.08	1.43	2955	9077	12026	4227
Romania	2575	3336	3066	2995	4.08	2.05	2.61	3.28	10497	6828	7987	9812
France	1764	1869	1848	1682	7.25	7.96	8.03	7.67	12797	14886	14843	12901
Italy	859	8554	927	911	7.26	8.66	8.66	8.71	6238	7394	8029	7937
Hungary	1154	1207	1121	1264	6.71	3.65	3.61	3.88	7745	4405	4044	4900
India	5781	6023	6258	6000	1.38	1.69	1.49	1.75	7983	10202	9348	10500
Indonesia	2909	3629	2940	3167	2.15	2.20	2.20	2.19	6256	7996	6460	6949
Thailand	1399	1236	1070	1200	2.71	2.97	2.71	3.17	3793	3672	2900	3800

Source: 1. FAO Quarterly Bulletin of Statistics, Vol. 8, 1995 p.20

2. Maize Board

Table 3: Provincial delimitation of area planted, maize production and mean yield in South Africa for the seasons 1992/93 to 1994/95 (Anonymous, 1995)

	Area Planted ('000 ha)									Production (kt)			Yield (t/ha)		
	1992/3			1993/94			1994/95			1992/3	1993/4	1994/5	1992/3	1993/4	1994/5
Province	White	Yellow	Total	White	Yellow	Total	White	Yellow	Total	Total	Total	Total	Total	Total	Total
Western Cape	1	1	2	1	1	2	1	2	3	2	5	6	1.102	1.925	1.873
Northern Cape	3	19	22	4	22	26	5	24	29	125	158	178	5.757	6.016	6.227
Eastern Cape	12	19	31	12	17	30	12	19	31	34	65	74	1.101	2.186	2.368
Free State	711	423	1134	727	529	1256	712	608	1319	850	3316	4334	0.750	2.640	3.285
KwaZulu/Natal	28	58	86	22	61	83	31	62	92	236	295	321	2.759	3.539	3.484
North-West	894	494	1387	1008	402	1410	1027	460	1487	405	2466	3618	0.292	1.749	2.434
Northern Province	36	8	45	35	12	47	34	10	44	48	69	95	1.072	1.453	2.150
Mpumalanga	134	495	629	113	539	652	150	594	744	1092	2254	2684	1.735	3.460	3.607
Gauteng	62	89	151	62	95	157	55	100	154	162	450	716	1.076	2.870	4.633
Total RSA	1881	1605	3487	1983	1680	3663	2027	1877	3904	2955	9077	12026	0.847	2.478	3.080

In the four years of production figures presented in Table 2, South Africa could only export grain in two years, importing grain in the other two years (Anonymous, 1995). Maize is primarily produced in the Highveld region of South Africa, although smaller production regions occur throughout the summer rainfall region (Table 3). White-grained maize is largely produced in the drier western regions of the country, whereas yellow-grained maize is predominantly produced in the eastern maize production regions. Maize farmers in KwaZulu/Natal produce the highest and most consistent yields under dryland conditions. This is as a result of the higher rainfall in this region. High yields realised in the Northern Cape are as a result of irrigation (Anonymous, 1989 and 1995).

The relative importance of the South Africa maize industry on the international market varies significantly. This is as a result of the variable climate and the large variation in the national yield. This can be seen in Table 2 where the area planted to maize stayed similar for 1991 to 1993, but the yield per hectare varied from 0.85 to 3.08 t ha^{-1} and total production from 2955kt to 12026kt. No other country has this kind of variation in grain yield. In terms of total production, South Africa ranks from thirteenth to fifth out of thirteen of the top maize producers (Anonymous, 1981, 1986, 1990 and 1995).

Many maize pathogens are present in South Africa, including fungi, bacteria and viruses (Gorter, 1977 and 1982). Most of these pathogens occur every season but are seldom of economic significance. During the 1970s and early 1980s, the leaf diseases (Gevers, 1975b; Le Roux, 1979; van der Watt, 1979; Kaiser and Nowell, 1983), stalk rots (Le Roux, 1975a; van der Watt, 1975a), common smut (le Roux, 1975b; van der Watt, 1975b) and head smut (Gevers, 1975a) were of concern to the maize industry. However, during the late 1980s *Stenocarpella* ear rot suddenly emerged as a pathogen of major economic importance in South Africa. A period of intense research into ear rot diseases and controlling the epidemic was undertaken (Rheeder, 1988; Gevers, 1989; Nowell, 1989a and 1989b; Rheeder *et al.*, 1989; Flett, 1990).

Losses due to ear rot, caused primarily by *Stenocarpella maydis*, were large in the 1986/87, 1987/88 and 1988/89 seasons. This can be see from the grading data presented in Table 4. The increase in Grades 2 and 3 over the previous, and subsequent, seasons was attributed solely to *Stenocarpella* ear rot. From Table 4 it can be seen that the problem occurred primarily in

yellow-grained hybrids. These hybrids are essentially planted in the higher rainfall production regions and this is where the ear rot epidemic was most intense.

Table 4: The proportion of each grade of maize delivered to the Maize Board from 1977 to 1995 (Anonymous, 1981, 1986, 1990 and 1995)

SEASON	% White Maize					% Yellow Maize				
	WM1	WM2	WM3	WM4	Sample	YM1	YM2	YM3	YM4	Sample
1976/77	65	30	5.1		0.1	77	22	1.3		-
1977/78	72	24	3.2		0.1	80	19	0.7		0.1
1978/79	68	30	2.2		-	85	15	0.3		-
1979/80	94	5	0.6		-	95	5	0.1		-
1980/81	81	17	1.5			88	12	0.2		
1981/82	79	18	2.8		0.1	81	19	0.4		0.1
1982/83	82	16	2.5		0.1	72	26	1.1		0.2
1983/84	88	10	1.4		0	83	16	0.4		0.1
1984/85	86	12	1.4		0	87	13	0.5		0
1985/86	93	6	0.7	0.3	0.1	84	15	0.7	0.1	0
1986/87	89	10	1.0			67	31	1.7	-	-
1987/88	81	15	3.8	-	-	40	44	15.7	-	-
1988/89	66	28	6.1	-	-	43	51	6.2	-	0
1989/90	93	7	0.4	0	-	87	13	0.2	-	-
1991/92	90	8	1.6			80	19	0.6		
1992/93	95	5	0.4			79	20	1.0		
1993/94	95	4	0.4			85	14	0.6		
1994/95	97	3	0.3		-	89	11	0.1		-
Mean	84	14	2.0			78	20	1.8		

The financial implications to the farmer and the maize industry as a whole were enormous. Unfortunately, this economic loss was never officially determined, but estimates have been placed around R200 million in 1986/87 alone (Viljoen[1], pers. comm.). Significant efforts were made to find effective control measures and to identify resistant maize hybrids by researchers in the public and private sector. Only in recent years are the research results on ear rot being published in more detail (Rheeder, 1988; Flett and Wehner, 1989; Gevers, 1989; Nowell, 1989a and 1989b; Flett, 1990 and 1991; Flett and Wehner, 1991; McLennan, 1991; Flett and

1 J.H. Viljoen, formerly Senior Manager: Product Services, Maize Board, P.O. Box 669, Pretoria 0001, RSA.

van Rensberg, 1992; Flett *et al.*, 1992; Gevers, *et al.*, 1992; Nowell, 1992; Ferreira, 1994; Flett and McLaren, 1994; Gevers and Lake, 1994; Gevers *et al.*, 1994; Bensch and Flett, 1995; Hohls *et al.*, 1995). As a result, significant progress had already been made in controlling ear rot by the early 1990s when grey leaf spot (GLS) first appeared.

Grey leaf spot (caused by *Cercospora zeae-maydis* Tehon & Daniels) first appeared in the midlands of KwaZulu-Natal in the 1988/89 growing season. From this initial focus the incidence of GLS spread rapidly, resulting in economic losses in commercial maize production during the 1991/92 season and emerged as a major threat to a significant part of the South African maize industry. The economic importance of this pathogen has increased in subsequent seasons. A significant amount of research has been conducted to accurately determine the effect the disease is having on the crop and to investigate various control measures. Initial estimates based on a single season's fungicide trials showed yield losses to be approximately 40% of the grain yield. This led to the rapid registration of fungicides to control the disease and the planting of less susceptible/more resistant maize hybrids (Ward *et al.*, 1993; Ward and Nowell, 1997).

Other maize diseases such as common rust (*Puccinia sorghi* Schw.) and northern leaf blight (*Exserohilum turcicum* [Pass.] Leonard & Suggs) are important (Kaiser and Nowell, 1983; Nowell, 1995). However, the importance of both *Stenocarpella* ear rot and GLS has increase out of proportion to the other diseases. For this reason, maize pathology research has been concentrated on these two diseases.

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SECTION I - EAR ROT

CHAPTER 1

A Review of *Stenocarpella* Ear Rot of Maize

ABSTRACT

A review of international literature shows that most research into *Stenocarpella* ear rot of maize has been undertaken in the U.S.A. and South Africa. There are a number of gaps in the understanding of the epidemiology of the two *Stenocarpella* pathogens and considerably more research has been undertaken on *Stenocarpella maydis* than on *S. macrospora*. These fungi are primarily debris and seed-borne pathogens with a limited host range. More information is needed on pycnidium and spore production on debris and subsequent dispersal methods. Information is limited on the adaptability of the fungi to various bioclimatic regions and the aggressiveness of isolates under different climatic conditions. Although the infection process of the fungi has been studied, the interaction between the infection and spread of the pathogen in the plant, and the environment is still not well understood, especially under stress conditions. There is considerable information on the genetics of resistance and on breeding methodology. Research has been undertaken on the effect of *Stenocarpella* colonised debris and resistance on the control of ear rot in maize but little is available on the effects of other agronomic and cultural practices.

1.1 INTRODUCTION

Grain yield and quality losses due to maize ear rots in South Africa have never been accurately quantified, but reports from other parts of the world have shown the losses to be very significant (Koehler, 1959; Shurtleff, 1980). The significance of ear rot in the South African maize industry, particularly *Stenocarpella* ear rot, increased significantly in the late 1980s. The estimated loss due to maize ear rot, primarily *Stenocarpella* ear rot, during the 1987/88 season was R200 million (Viljoen[2], pers. comm.) and the severity of ear rots was not as great as in 1988/89 season (Table 1.1). Compared to the 1986/87 season, the disease was much more widespread in these two seasons; i.e., the incidence of ear rots in the western maize

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[2] J.H. Viljoen, formerly Senior Manager: Product Services, Maize Board, P.O. Box 669, Pretoria 0001, RSA.

producing region increased significantly rather than being limited to the eastern maize producing regions (Viljoen, pers. comm.).

An overall summary of the grain grade composition of the maize crop from 1980/81 to 1993/94 is presented in Table 1.1.

Table 1.1: Grade composition (%) of the annual South African maize crop from 1980/81 through 1993/94 (Viljoen, pers. comm.)

Marketing Season	Yellow Maize			White Maize		
	YM1	YM2	YM3	WM1	WM2	WM3
1980/81	88	12	0.2	81	17	1.5
1981/82	81	19	0.4	79	18	2.8
1982/83	72	26	1.1	82	16	2.5
1983/84	83	16	0.4	88	10	1.4
1984/85	87	13	0.5	86	12	1.5
1985/86	84	15	0.7	93	6	0.7
1986/87	*67*	*31*	*1.7*	*89*	*10*	*1.0*
1987/88	*40*	*44*	*15.7*	*81*	*15*	*3.8*
1988/89	*43*	*51*	*6.2*	*66*	*28*	*6.1*
1989/90	87	13	0.2	93	7	0.4
1990/91	73	25	1.2	88	10	2.0
1991/92	81	19	0.6	90	9	1.5
1992/93	80	19	1.0	95	5	0.5
1993/94	85	14	0.5	95	4	0.4

YM1 & WM1 = up to 4% by mass of discoloured and/or defective grain.
YM2 & WM2 = between 4 and 8% by mass of discoloured and/or defective grain.
YM3 & WM3 = greater than 8% by mass of discoloured and/or defective grain.

In order to control ear rot of maize it is essential to understand the organisms involved and to understand the large number of environmental, agronomic and genetic factors affecting this disease complex. Maize ear rot fungi are an integral part of a complex of pathogens responsible for the seedling, root, stalk and ear rot diseases of maize.

Diplodia maydis (Berk.) Sacc. and *D. macrospora* Earle were renamed *Stenocarpella maydis* and *S. macrospora*, respectively, in 1980 by Sutton. However, general acceptance of this change in name may take time since these fungi have been known as *Diplodia* species since 1848 (Sutton and Waterston, 1966b). Of the two *Stenocarpella* species that cause both stalk and ear rots of maize, the more common is *S. maydis*. Although *S. macrospora* is less common, the fungus is more aggressive than *S. maydis* and can cause severe losses (Latterell and Rossi, 1983).

Synonyms for *S. maydis* are *Sphaeria maydis* Berk., *Sphaeria zeae* Schw., *Diplodia zeae* (Schw.) Lev., *Hendersonia zeae* (Schw.) Hazsl., *Macrodiplodia zeae* (Schw.) Petrak and Syd., *Phaeostagonosporopsis zeae* (Schw.) Woron., *Diplodia maydicola* Speg., and *Diplodia zeae-maydis* Mechtij (Sutton and Waterston, 1966b; McGee, 1988). The disease is commonly known as Diplodia ear rot or dry rot (McGee, 1988). The pycnidia are immersed, spherical to subglobose, dark brown to black, 150 - 300 μm in diameter. The pycnidial wall is multicellular and darker round the circular protruding papillate ostiole which is 40 μm in diameter. The conidia are straight, curved or irregular, 1 (0-2) septate, smooth-walled, pale brown, apex attenuated or rounded, base truncate, 15 - 34 x 5 - 8 μm, formed from hyaline, aseptate, cylindrical phialides, 10 - 20 x 2 - 3 μm. Scolecospores have been reported (Sutton and Waterston, 1966b). On occasion pycnidia are colourless with long, narrow, scolecospores, 1 - 2 x 25 - 35 μm in size. No teleomorph is known (Shurtleff, 1980).

Synonyms for *S. macrospora* are *Macrodiplodia macrospora* (Earle) Hohnel, *Macrodiplodia zeae* (Schw.) Petrak and Sydow var., *Diplodia macrospora* (Earle) Petrak and Sydow, and *Stenocarpella zeae* Sydow. Common names for the disease, caused by this fungus, include Diplodia ear rot and dry rot (Sutton and Waterston, 1966a; McGee, 1988). Pycnidia are immersed or superficially embedded in agar, 180 - 360 μm in diameter, solitary or in large, stromatic groups up to 2 mm or more in diameter, carbonaceous, covered with white to yellowish-brown mycelium, ostiolate, becoming erumpent at maturity to discharge conidia in dark brown to black droplets or cirrhi. Conidiogenous cells arise from the inner cell wall tissue of the pycnidia and are short, hyaline, aseptate, cylindrical and are 6 - 13 x 2.5 - 4.0 μm in size. Conidia are cylindrical to clavate with a rounded apex and truncate base, straight or slightly curved, brown, 0- to 3-septate, not or slightly constricted at the septa and 55 - 106 x 6 - 11 μm in size. Scolecospores (first reported by Hoppe, 1943) are formed in pycnidia on

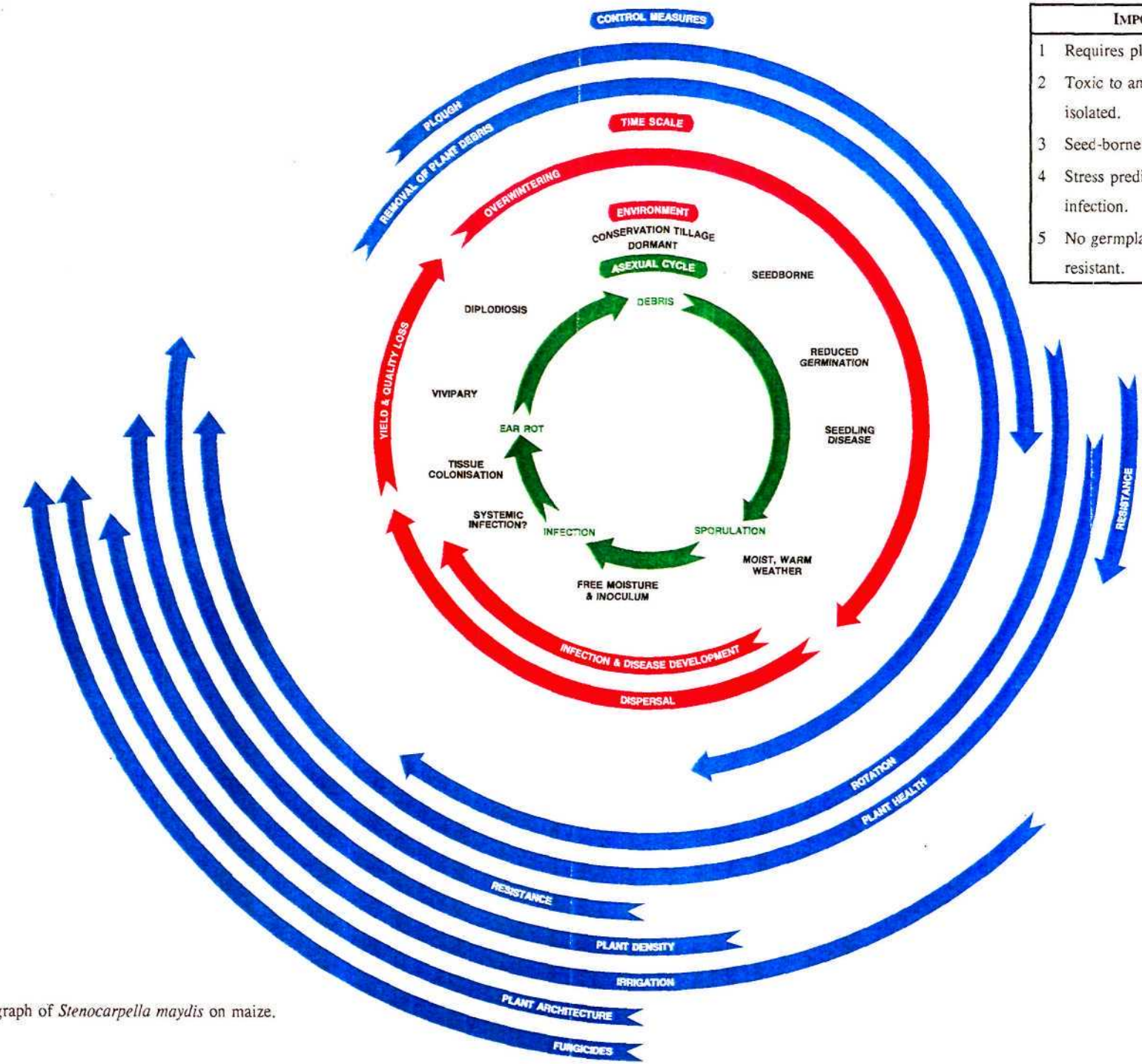
infected maize kernels and are extruded in cream-coloured droplets or cirrhi, and are filiform or sperm-shaped, hyaline, aseptate, 20 - 40 x 1.2 - 1.0 μm in size (Marasas and van der Westhuizen, 1979).

S. macrospora is readily distinguishable from *S. maydis* by the large, 0- to 3-septate conidia, as well as by its requirement for biotin (first reported by Wilson, 1942) when cultured on synthetic media (Sutton and Waterston, 1966a; Morant *et al.*, 1993).

The aim in plant pathology is to arrive at a clear understanding of each disease triangle or quadrangle studied (Robinson, 1976). The function of each contributing component studied is unimportant until integrated into the overall picture. This becomes difficult when a considerable amount of information is known about the disease. The concept of an ethograph (Putter, 1980) can be used to integrate and simplify information available on the disease quadrangle concerned. An ethograph is a graphical integration of epidemiological information. The ethograph starts from a central core of information and is built in a series of concentric spheres of information covering each systems level, from the molecular system in the middle to the population system on the outside. This concept has been taken and ethographs generated for *S. maydis* (Figure 1.1) and *S. macrospora* (Figure 1.2).

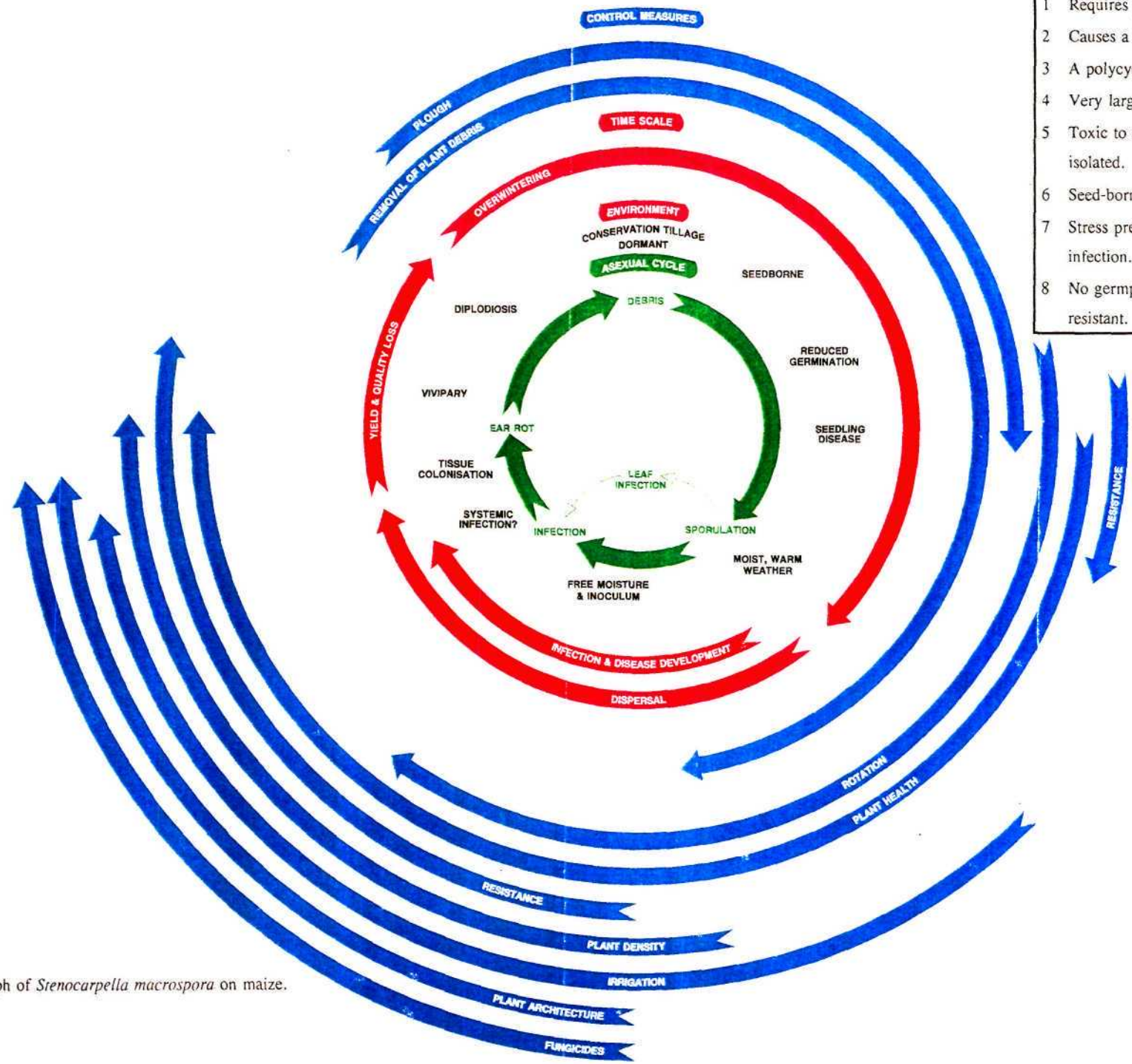
The net value of the ethograph is that it enables one to examine all contributing factors of a disease in one figure. The similarities and differences can be compared and identified. Based on the understanding on the key components of an ethograph, a series of intervention points can be identified at which disease control measures could be applied (Laing, 1987). The importance of each intervention point is related to the quantitative contribution of each factor in the epidemic.

This chapter follows the sequence depicted in the ethographs in Figures 1.1 and 1.2.



Important Points	
1	Requires plant debris for survival.
2	Toxic to animals but no mycotoxin isolated.
3	Seed-borne and seed-transmitted.
4	Stress prediposes plants to ear rot infection.
5	No germplasm is completely resistant.

Figure 1.1: Ethograph of *Stenocarpella maydis* on maize.



1 Requires plant debris for survival.
2 Causes a leaf blight.
3 A polycyclic pathogen.
4 Very large conidia.
5 Toxic to animals but no mycotoxin isolated.
6 Seed-borne and seed-transmitted.
7 Stress prediposes plants to ear rot infection.
8 No germplasm is completely resistant.

Figure 1.2: Ethograph of *Stenocarpella macrospora* on maize.

1.2 Crop Residue and Tillage Practices

S. maydis overwinters as conidia in pycnidia on debris and as conidia or mycelium on maize seed. Under warm, moist conditions, conidia are extruded from the pycnidia in long cirrhi and are disseminated by rain, wind and insects (Shurtleff, 1980).

S. maydis ear rot increases significantly with increased surface debris through reduced tillage practices (Kerr, 1965; Palti, 1981; Flett, 1990; Flett and Wehner, 1991). Field sanitation is important in reducing maize ear rot inoculum for the following maize crop (Koehler, 1959; Kerr, 1965; Flett, 1990; Flett and Wehner, 1991; Flett *et al.*, 1992). Latterell and Rossi (1983) expressed concern over the potential for a *S. macrospora* epidemic to develop as a result of increased conservation tillage in the more humid regions of the U.S.A. Johansen (1987) summarised the problem as follows: "No-till and reduced tillage offer many benefits to the farmer. Unfortunately, these options appear to have a hidden cost in increased opportunity for plant diseases". *S. maydis* overseasons in plant debris and there is no reason why it should not survive for a number of years in this manner (Eddins, 1930). It is important to incorporate maize debris thoroughly as conidia from pycnidia of *S. maydis* on stalk debris have been found to be viable after lying in a clover field for two years (Koehler, 1959). In South Africa, Flett (1990) and Flett *et al.* (1992) showed that colonised maize debris on the soil surface survived for longer, had an increased pycnidial density with greater conidial viability, and an enhanced re-isolation frequency of its pathogens, when compared to colonised debris buried below the soil surface for up to 11 months. The decline in fungal survival was greatest once the summer rains had begun. Flett (1990) showed that *S. maydis* and *S. macrospora* cannot colonise maize debris saprophytically. Flett and Wehner (1991) found that reduced-tillage practices significantly increased the incidence of *S. maydis* ear rot over two sites and two seasons in South Africa. This can be seen in Tables 1.2 and 1.3. A further four localities showed the same trends but they were not significantly different from the ploughed treatment. Although only present at one site, *S. macrospora* was not significantly influenced by tillage practices.

Table 1.2: The incidence of *Stenocarpella* spp. on maize grown under different tillage systems at Geluksburg (Flett and Wehner, 1991)

Tillage	% Sten ears		% Rot kern.		% Smay kern.	
	*87/88	88/89	*87/88	88/89	*87/88	88/89
No Tillage	35.4a	8.5a	47.0a	20.8a	53.0a	20.7a
Chisel	15.3b	-----	45.8a	-----	27.1ab	-----
Chisel X2	13.7b	5.8b	37.9ab	15.7ab	25.8ab	10.9b
Chisel:Disc	-----	4.1b	-----	8.9b	-----	5.4b
Plough:Disc	7.8c	2.9b	32.b	10.9b	11.4b	5.0b

* = log transformation
Means followed by the same letter are not significantly different at the 5% level (SNK test for significant differences)
% Sten ears = Percent ears infection by *Stenocarpella* spp.
% Rot kern. = Percent rotten kernels determined by mass.
% Smay kern. = Percent kernels infected by *S. maydis*.
----- = tillage treatment not included in the trial that season.

Table 1.3: The incidence of *Stenocarpella* spp. on maize grown under different tillage systems at Bloekomspruit (Flett and Wehner, 1991)

Tillage	% Sten ears		% Rot kern.		% Smay kern.	
	*87/88	88/89	*87/88	88/89	*87/88	88/89
No Tillage	47.5a	20.3a	47.3a	21.5a	52.7a	25.3a
Chisel	45.9a	16.2a	39.6a	23.2a	47.3a	25.8a
Chisel:Disc	39.5a	8.8b	37.6a	18.6a	43.2a	27.2a
Plough:Disc	24.0b	5.0b	23.0b	7.0b	25.3b	6.3b

* = log transformation
Means followed by the same letter are not significantly different at the 5% level (SNK test for significant differences)
% Sten ears = Percent ears infection by *Stenocarpella* spp.
% Rot kern. = Percent rotten kernels determined by mass.
% Smay kern. = Percent kernels infected by *S. maydis*.

1.3 Crop Rotation and Sanitation

Monoculture is not detrimental to the yield of all crops and will not always increase disease. However, in maize it is usually detrimental to grain yield and results in increased disease levels (Shipton, 1977; Palti, 1981). Koehler (1959) suggested that rotation, especially with soybeans, results in increases in grain yield and decreased the prevalence of stalk and ear rot. Increases in ear rots occurred after the second consecutive year of maize cultivation. Kerr (1965) found that there were significant reductions in the incidence of both *S. maydis* and maize stalkborer

(*Busseola fusca* Fuller) when a greencrop was planted between maize crops. Crop rotation not only decreases disease, but can increase yield and hold down costs of fertilizer and herbicides in maize (Koehler, 1959; Shipton, 1977; Palti, 1981; Sumner *et al.*, 1981; Reagan, 1989). Reagan (1989) suggested not only the rotation of crops, but also the rotation of varieties, tillage methods and pesticides. This allows the farmer to obtain maximum benefit from all factors that can be varied. The rotation of crops is widely practised in countries with developed economic and agricultural infrastructure; i.e., first world countries. However, South Africa does not fall into this category (Channon and Farina, 1991).

1.4 Hosts and Distribution

Both species of *Stenocarpella* are widely distributed. *S. maydis* is known to occur in Africa, Asia, Australasia, Europe, North America and South America. *S. macrospora* was first noted in 1928 during a survey undertaken in Florida, USA (Eddins, 1930) and the fungus is widespread in Africa, Asia, Australasia, Europe, North America, Central and South America and the Caribbean (Sutton and Waterston, 1966a). *S. macrospora* is of more limited distribution than *S. maydis*, being confined largely to the warmer areas (Stevens and Chapman, 1942) and of less economic significance than *S. maydis* in the USA. *S. macrospora* is of more economic importance in Central America (McGee, 1988), being more prevalent in the tropics, because this species needs higher temperatures than *S. maydis* to manifest itself (Stevens and Chapman, 1942; Sutton and Waterston, 1966a and 1966b). *S. macrospora* was first noted in Zimbabwe in 1955 (Kerr, 1965). In South Africa, its distribution is limited to the provinces of Mpumalanga, eastern Free State, Eastern Cape and KwaZulu-Natal (Marasas and van der Westhuizen, 1979; Rheeder, 1988; Rheeder *et al.*, 1989; Flett, 1990; McLennan, 1991; Rheeder and Marasas, 1994).

The only known hosts of *S. maydis* are *Zea mays* L. and *Arundinaria* spp. (Sutton and Waterston, 1966b; Flett, 1991).

1.5 Infection of the Host, Symptoms and Disease Development

1.5.1 Infection and Symptoms

Ears are most susceptible in the period between silk emergence and approximately three weeks after silking, and hybrids with thin pericarps are often very susceptible (Shurtleff, 1980). However, ear infection can occur any time from silking until the grain and cob become too dry (normally below 22% grain moisture). *S. maydis* infection at the base of the ear and the shank area of the ear leads to ear infection (Durrell, 1923; Clayton, 1927; Eddins, 1930; Palm and Calvert, 1981). Inoculation studies by Koehler (1959) suggested that the fungus may move from the stalk into the ear. Although the fungus could not be traced during the growing season, inoculation of both *Stenocarpella* species into the internode below the ear shortly after anthesis, resulted in a significant increase in ear rot at harvest (McLennan, 1989 and 1991). Bird and insect feeding on the ear tips increases the potential for ear rot (Koehler, 1959; Shurtleff, 1980).

Initial infection takes place at the stalk-attached end of the ear and then ramifies through the ear towards the tip (Manns and Adams, 1923; Clayton, 1927; Koehler, 1942; Ullstrup, 1949; Koehler, 1959; Shurtleff, 1980; Bensch, 1994 and 1995b). Bensch (1995b) showed that infection of the ear starts with the colonisation of the pedicel and embryo region of the kernels. Infection takes place before black layer formation, allowing for rapid colonisation of the grain. Pith tissue is colonised once the entire cob (sclerenchyma and placenta tissues) is heavily infected with *S. maydis*. Higley *et al.* (1993) found it difficult to isolate *S. maydis* from the core of the cob. Light and electron microscopy have shown that infection of the seed by *S. maydis* takes place at the base of the seed. Then the regions between the embryo and pericarp, and the embryo and endosperm are colonised (Achar and Rabikoosun, 1995). Eddins (1930) suggested primary infection to be through the tip of the ear.

The husks of early-infected ears appear bleached or straw-coloured. If infection occurs within two weeks of silk emergence, the entire ear turns greyish brown, is shrunken, lightweight and completely rotted. These ears stand upright with the inner husks adhering tightly to one another or the grain because of mycelial growth between them. Black pycnidia may be scattered on the husks, floral bracts, cob tissues and the sides of kernels. When the husks of

colonised ears are opened, a white mould is visible which starts at the base of the ear and spreads towards the tip. Ears infected later in the growing season show no external symptoms, but when the ears are broken and kernels removed, a white mould is commonly encountered growing between the kernels, the embryos / tips of which are discoloured. Some isolates cause vivipary (premature germination) (Shurtleff, 1980).

In South Africa, ear infection by *S. maydis* has been most closely correlated with high night temperatures and total rainfall. Conidia were found to germinate only in the dark (McLennan, 1991). It has been shown that *S. maydis* conidia can be airborne and remain viable under cool temperatures and reduced humidity (Flett and Wehner, 1989).

Infection of seeds has been shown to be as high as 66.7% in the USA and 38% in Nigeria (Latterell and Rossi, 1983; McGee, 1988), but has not been shown to be seed-transmitted (McGee, 1988). Seed-borne inoculum can cause seedling blight (Koehler, 1959; Shurtleff, 1980; Rheeder, 1988; McLennan, 1991) but does not appear to occur very frequently (Kerr, 1965). In tests conducted by Nwigwe (1974), it was shown that *S. maydis* infection of seed could cause a reduction in germination of between 5 - 37%. Rheeder (1988) and Rheeder *et al.* (1990) showed that seed-borne infection of both *S. maydis* and *S. macrospora* had a negative effect on germination on maize in South Africa, with *S. maydis* having the larger effect.

S. macrospora is usually first apparent as a leaf blight, with the crop growth stage at initial infection dependent on the environment. Initially lesions are grey-green, elliptical, 3-5 mm in diameter and water-soaked in appearance. Later these necrotic lesions may be 10cm in length, with pycnidia forming in the centre (Latterell and Rossi, 1983). In laboratory studies, leaf infection did not take place below 15°C (McLennan, 1991). Macroscopically, the symptoms of ear rot of both *Stenocarpella* species are very similar but *S. macrospora* is much more aggressive than *S. maydis*. *S. macrospora* often infects the ear primordia behind the leaf, spreading rapidly, and causing sheath necrosis that results in the progressive death of the leaf blades (due to lack of water translocation) while the fungus spreads up the stalk. *S. macrospora* usually results in the blighting of the ear leaf blade and death of the leaf, which is not the case for *S. maydis*. Under high humidity, more severe and heavy mycelial growth appears on the stalks and ear husks. *S. macrospora* pycnidial production on the stalks is

greater than that of *S. maydis*, but it is lower on the ear husks (Latterell and Rossi, 1983).

S. macrospora conidia germinate over a temperature range of 5-39°C and germinate in water within 16 hours at 25-32°C, with each of the cells able to produce a germtube (Eddins, 1930). Spore germination can take place in the dark or light (McLennan, 1991), in contrast to *S. maydis* (dark only).

McLennan (1991) showed that *S. macrospora* incidence in South Africa was most closely associated with three environmental parameters:

a) the number of days with rain per month,
b) the number of days with mist,
c) the daily maximum temperature.

These data suggest that hot, dry weather would limit the spread of the pathogen to a large part of the maize production region in South Africa.

At least 25 biotypes of *S. maydis* are known to exist (Kappelman *et al.*, 1965; Sutton and Waterston, 1966b) and physiological specialisation is not known to occur in *S. macrospora* (Sutton and Waterston, 1966a).

Rheeder (1988) and Rheeder *et al.* (1990) found that there was an inverse association between *S. maydis* and *S. macrospora* in individual kernels in South African maize. This effect was first reported by Hoppe and Holbert (1936) in the USA. These fungi were also inversely associated with the Fusaria (Rheeder, 1988; Rheeder *et al.*, 1990). Although *S. macrospora* produces fewer conidia than *S. maydis* per pycnidium, the polycyclic nature (production of pycnidia on leaf lesions before ear emergence) of *S. macrospora* allows for repeated leaf and ear infection to take place when climatic conditions are suitable (McLennan, 1991).

Koehler (1959) found that the stage of maturity of the crop at harvesting can be highly significant in reducing the percentage of the crop that is rotten. Very significant reductions in the percentage of diseased kernels occurred when the maize was harvested early in 9 of 11 years. There appeared to be no further ear rot development below 21 % grain moisture in the field, but the relationship between kernel moisture above 21% and ear rot prevalence was low. The reason for increased ear rots in the late harvested maize was the amount of rainfall late

in the season.

1.5.2 Mycotoxins

Initial studies by van der Bijl (1916) indicated that *S. maydis*-infected maize had no effect when fed to cattle. However, in 1918 paralysis was reported in cattle (later called diplodiosis) when they grazed in lands containing *S. maydis*-infected ears (Mitchell, 1918). Diplodiosis is now known to occur in South Africa, Zimbabwe and Zambia. Field outbreaks of diplodiosis usually occur during late, wet seasons, when cattle and sheep graze harvested maize fields. Horses, donkeys and mules grazing the same fields are unaffected. Diplodiosis has been reproduced experimentally by feeding cattle and sheep either maize ears naturally infected with *S. maydis*, or pure cultures grown on autoclaved maize. No response has been reported in feeding experiments with almost identical feed material to mules, horses, goats and pigs. Sensitivity of animals within species has been shown to vary considerably (Marasas, 1977a, 1977b and 1977c). Economic losses are readily incurred by the poultry industry should poultry feed be contaminated with *S. maydis*. Broilers, ducklings and laying hens are very sensitive to maize colonised with *S. maydis,* with as little as 5% infected maize in the feed resulting in significantly poorer weight gains and egg laying. Ducklings appear to be the most sensitive to diplodiosis (Rabie *et al.*, 1987; Rheeder, 1988).

In cattle, diplodiosis can occur from a few days to two weeks after they start grazing mouldy ears. Apparently healthy cattle can become ill up to 10 days after removal from the contaminated food source. The first symptoms are lachrymation and salivation accompanied by quivering of the shoulder and flank muscles, and ataxia. With time the clinical signs are more conspicuous; the back is arched, muscular tremors become general and marked ataxia develops. Movement is restricted to a minimum and then signs of poor co-ordination are shown as high stepping, lateral swaying and a tendency to walk with hind quarters bent to one side. Cattle that continue to feed on infected maize will exhibit more pronounced symptoms and muscular paralysis sets in, with death following. If the animals are removed from the contaminated food source when clinical signs become evident, a rapid recovery is made with signs of paralysis disappearing within a few days. Gross pathological changes are not normally associated with diplodiosis (Marasas, 1977b; Kellerman *et al*, 1985).

The toxin diplodiol (trans-6-ethyl-5-hydroxymethyl-5,6,7,8-tetrahydrochromone) was isolated from *S. macrospora*, which was believed to be responsible for maladies in chicks. This included heart and liver enlargement, effusion of major organs, rupturing of blood vessels in the skin and premature death. A further toxic compound to chicks, chaetoglobosin K, was found to be produced by *S. macrospora* and this compound also appears to cause selective growth responses in plants (Cutler *et al.*, 1980a and 1980b).

1.6 Host Resistance

Resistance breeding is a complex topic that involves plant physiology, morphology, genetics, environmental factors and specialised manipulative genetic breeding techniques. Resistance breeding frequently has to make use of artificial inoculation techniques and subsequent specialised rating techniques to differentiate between plant genotypes.

Pappelis *et al.* (1973) found that there was a relationship between pith parenchyma cell death and infection by *S. maydis* in the ears of maize. Cob pith condition determined whether or not the cob could be invaded by *S. maydis*. A hybrid with a slow death rate of the cob pith restricted the growth of the fungus severely, when compared to a hybrid with a rapid cell death rate of the cob pith.

Hooker (1956) found that there was significant variation in resistance of maize inbreds to *S. maydis*. There is also great variation in the incidence of the ear rots and resistance levels between years (Thompson *et al.*, 1971). Koehler (1959) found that there was superior germplasm against ear rots, but generally this material did not have the necessary yield characteristics. Wiser *et al.* (1960) studied six inbreds, by artificially inoculating with *S. maydis*, and found that ear rot resistance was quantitatively inherited, but partial dominance played a role. There was apparently no inbred that was completely resistant to the ear rot fungi, although there were high levels of resistance available (Koehler, 1959; Kerr, 1965). In contrast, high lysine maize in the USA was found to be hyper-susceptible to *S. maydis*, *Gibberella zeae* (Schw.) Petch. and especially *Fusarium moniliforme* Sheld. This trend followed through from the inbreds to the hybrids but was dependent upon the background of the material that the Opaque-2 gene was introduced into (Ullstrup, 1971). In South Africa, high lysine hybrids and inbreds have been developed that have significant levels of resistance

to *S. maydis* ear rot (Gevers, 1989; Gevers *et al.*, 1992). In an analysis for *S. maydis* ear rot resistance across all inbred-types, Gevers *et al.* (1992) found that there were significant levels of ear rot resistance throughout their inbred nurseries. There were significant differences in susceptibility between heterotic groups of inbreds, and between yellow grain, white grain and opaque-2 inbreds. Of the yellow grain inbreds, the heterotic groups F and M exhibited significantly more ear rot resistant inbreds than did other heterotic groups. The R heterotic group (Reid) was characterised by a higher frequency of highly susceptible inbreds. The white-grained inbreds had a greater number of inbreds resistant to ear rot than the yellow-grained inbreds. Ear rot resistance was most frequent and highest in the F heterotic group. The opaque-2 maize inbreds showed a range in response to ear rot, with most showing resistance. A significant number of inbreds showed a high degree of resistance, particularly in the F and M heterotic groups. These trends could be seen in the ear rot response of commercial maize hybrids in South Africa. B73-type parents, and to a less extent I137Tn, are blamed for the significant susceptibility to *Stenocarpella* ear rot in specific yellow-grained commercial hybrids (Gevers *et al.*, 1992).

A diallel analysis of inbreds for ear rot resistance to *S. macrospora* by McLennan (1991), showed resistance was mainly additive in nature, but dominance was significant at times. D940Y exhibited a high specific combining ability for ear rot resistance. Epistasis in resistance was also present in a number of inbreds. This meant that both recurrent selection and backcrossing could be used to improve *S. macrospora* ear rot resistance of susceptible inbreds, depending upon the resistance source (McLennan, 1991).

Significant differentiation between hybrid responses to *Stenocarpella* ear rot infection (assumed to be *S. maydis*) has been demonstrated in South Africa. Although there is a complex interaction with the environment, differentiation is possible between hybrids. This allows for reliable recommendations to be made to farmers regarding the relative susceptibility of hybrids to *S. maydis* ear rot infection. In general terms, most studies have shown that white-grained hybrids are more resistant to ear rot than yellow-grain hybrids (Rheeder, 1988; Gevers, 1989; Nowell, 1989 and 1992; Rheeder *et al.*, 1989; Rheeder and Marasas, 1992; Ferreira, 1994; Flett and McLaren, 1994; McLaren and Flett, 1994; Rheeder and Marasas, 1994). Rheeder (1988) and Rheeder and Marasas (1992) found significant differences between commercial hybrids in South Africa in their response to *S. macrospora* ear rot.

Nowell (1992) suggested that breeding strategies for ear rot resistance be modified to deal with the increased inoculum pressure resulting from conservation tillage practices. Such modification would include increasing the inoculum pressure in breeding nurseries, an increased emphasis on ear rot resistance, improving the screening of inbreds and hybrids for ear rot resistance, and improving ear rot assessment methods. Cognisance needed to be taken that *Stenocarpella* ear rot is a portion of the root, stalk, ear and seedling disease complex of maize. A range of ear rot fungi can cause stalk, root, ear and seedling diseases of maize under ideal conditions. Some ear rot pathogens can also causes a leaf blight. Environmental conditions and interactions with the other pathogens will largely influence the type of ear rot and the build up in inoculum in the crop residue (Koehler, 1959; Koehler, 1960).

1.6.1 Inoculation Techniques and Selection Methods

Ullstrup (1949) was the first to inoculate maize artificially by spraying the ears with a conidial and mycelial fragment suspension. The inoculated ears had to be covered to prevent rapid desiccation of the suspension. This technique resulted in the successful differentiation of ear rot resistance between genotypes. Effective inoculation required selection of isolates that were aggressive and sporulated profusely, and the best growth stage for inoculations was found to be from anthesis to approximately three weeks after pollination (Ullstrup, 1949; Koehler, 1959; Ullstrup, 1970; Chambers, 1986 and 1988). Koehler (1959) found the most susceptible period for the maize ears was from silking to between 20 and 40 days after silking. However, he also found that inoculation can still result in more than double the natural amount of ear rot when inoculated 60 days after silking. In Zimbabwe, Kerr (1965) tried inoculating *S. maydis* using an injection method, spraying the silks and by introducing a disc of agar culture under the ear sheath. Kerr concluded that there appeared to be a lack of correlation from year to year when the pathogen was introduced artificially into the ear, regardless of the method employed. Villena (1969) found that spraying inoculum on the ears to induce disease should be done within 10 days of mid-silk. He also noted that the fungus needed 10 - 20 days from the time of application to penetrate the ear husk. By introducing the inoculum into the tip of the ear, the infection level was almost 100% when inoculated from mid-silk to 20 days post-silk. Ullstrup (1971) found that the incidence and severity of *S. maydis* ear rot was directly proportional to the spore concentration in the inoculum. A conidial concentration of 1.2×10^6 conidia ml^{-1} gave a mean of 81.5% infected ears but a dilution of 1000 resulted in only 9.1%

infected ears. McLennan (1991) found no significant increase in ear rot when dipping the silks in a *S. maydis* or *S. macrospora* conidial suspension or when introducing conidia into the tip of the ear.

Villena (1969) found that the inoculation of *S. maydis* covered toothpicks (a technique developed by Young, 1943) up to 10 days after mid-silk resulted in completely rotten ears. The optimum time for inoculation appeared to be 10 - 30 days after mid-silk, as inoculation later than this resulted in low levels of ear infection. Villena suggested rating the ears about 15 days after toothpick inoculation. Chambers (1988) tested the toothpick method of introducing *S. maydis* into the ears in South Africa. He found the optimum time for inoculation to be 3 - 4 weeks after mid-silk. The severity of rot was similar when the ears were inoculated up to 16 days post mid-silk. However, there was a significant decrease in the severity of rot when the ears were inoculated from 16 - 24 days post mid-silk and a rapid decrease when the ears were inoculated 24 days post mid-silk. The incidence of ear rot when the ears were inoculated 16 days after mid-silk was so severe that differences between genotypes could not be determined. Inoculation four weeks after mid-silk resulted in the incidence being so light that there was not enough disease to test resistance. The rate of infection or susceptibility decreased rapidly with grain moisture levels below 66%. Rheeder and Marasas (1992) noted that significant discrepancies arose between hybrid ear rot response to natural infection of *S. maydis*, and hybrids artificially inoculated either in the tip or shank of the ear.

Warren and Onken (1981) showed that effective ear rot epidemics could be induced by applying a 3ml spore suspension (40 000 conidia ml^{-1}) of *S. maydis* to the leaf whorl of the plants, 2 weeks prior to anthesis. The percent rotten ears was determined 70 days after inoculation. This method resulted in good differentiation in ear rot resistance between 50 maize hybrids. Results obtained were comparable to field infection levels. Klapproth and Hawk (1991) compared depositing a spore suspension of *S. maydis* into the sheath cavity, placing infected popcorn in the whorl, spraying a conidial suspension on the silks, and injecting a conidial suspension directly into the ear, using both inbreds and hybrids. The first two techniques resulted in low levels of infection, whereas the direct placement of the inoculum into the ear resulted in severe infection. The most suitable method was that of spraying the silks with a conidial suspension. This method resulted in good differentiation between

genotypes and was easy to apply. This was shown to be true under South African conditions (McLennan, 1991).

Inoculation in the leaf whorl with milled *Stenocarpella*-rotted ears, from the previous growing season, was first undertaken by Nowell (1989). Subsequent studies have refined the technique, suggesting that 3.5 g of milled inoculum needs to be applied to the whorl of each plant, 10 -14 days before anthesis. This will result in significantly more ear rot than non-inoculated maize (Nowell, 1992). A study in South Africa showed that placing a conidial suspension behind the ear shank resulted in a significant correlation in hybrid response to ear rot with the natural disease incidence over two seasons. However, for the evaluation of maize genotypes for resistance to ear rot, it was recommended that milled infected maize kernels be placed in the whorl at three growth stages. This frequency of application would be determined by the quality and viability of the inoculum used i.e. whether infected ears from the field or pure cultures of *S. maydis* produced in the laboratory were used (Bensch, 1995a). Ideally, inoculum should be produced as pure fungal culture on maize that is milled before application (Flett and McLaren, 1994).

Morant *et al.* (1993) developed a growth medium to optimise the production of conidia for both *S. maydis* and *S. macrospora* while keeping the mycelial growth to a minimum. This allows for the production of large numbers of conidia and easy preparation of a conidial suspension.

1.6.2 Ear Rot Assessment

Hoppe and Holbert (1936) reported ear rot as a percentage of ears colonised with *S. maydis*, or the percentage of diseased grain by weight of infected kernels in a representative sample of 250 g of shelled maize from the entire plot. All three methods of ear rot assessment were satisfactory, although the correlation between the percentage rotten ears and the percentage diseased kernels (by weight) was not always good. This was due to the high variation in natural infection with an experiment and the amount of concealed infection present on the ears (ear rot not visible before shelling). After artificial inoculation, the percentage diseased ears was a satisfactory measure, especially when the ears were categorised into the various groups of disease severity (Hoppe and Holbert, 1936). Koehler (1959) found that ear rot determinations could be made by expressing the number of rotted ears as a percentage in the

years when there was no concealed *S. maydis*. In years with concealed disease, it was necessary to determine the percentage rotted kernels by weight from samples of between 200 and 500 g. One person should undertake the diseased kernels determination for a particular experiment as there can be significant differences in the rating of samples by different people. Some of the ear rot assessment methods are summarised in Table 1.2.

Gulya *et al.* (1980) showed that the ear rot rating means, when using a scale, are logarithmic in nature and cannot easily be converted to arithmetic means. This often results in misleading comparisons being made as can be seen from Table 1.3. This problem can be eliminated through the use of a linear scale of 1 to 100 which is represented by the upper limit of each category (1 = 0 - 1%, 10 = 1 - 10%, 25 = 10 - 25%, 50 = 25 - 50%, 100 = 50 - 100%). The increments in this scale are proportional to the actual percentages they represent. In addition, the use of the upper limits can be justified by the fact that any overestimation will allow for symptomless infection.

Table 1.2: Summary of maize ear rot rating scales reported in the literature

Index	Villena (1969)	Pappelis *et al.* (1973)	Gulya *et al.* (1980)
0			
0.5		0 to 12% of the pith discoloured	
1.0	0% ear infected	13 to 25%	0-1% rotten ears
1.5			
2.0	25% ear infected	26 to 50%	1-10% rotten ears
2.5			
3.0	50% ear infected	51 to 75%	10-25% rotten ears
3.5			
4.0	75% ear infected	76 to 100%	26-50% rotten ears
5.0	100% ear infected		50-100% rotten ears
6.0	100% ear infected with heavy accumulation of cottony mycelium		
7.0	100% infected with ear completely rotted or premature death		

Table 1.3: Maize ear rot scores of three hypothetical rows, using a non-linear 1-5 scale with the corresponding linear 1-100 scale ratings in parentheses (Gulya *et al.*, 1980)

Row	Individual ear scores					Mean	% Actual rot
1	1 (1)	1 (1)	3 (25)	5 (100)	5 (100)	3.0	45
2	3(25)	3 (25)	3 (25)	3 (25)	3 (25)	3.0	25
3	4(50)	4 (50)	4 (50)	4 (50)	3 (25)	3.8	45

The ear rot epidemics in South Africa during the 1986/87 and 1987/88 seasons caused South African pathologists to seek more accurate methods of ear rot assessment and presentation. Depending upon the accuracy desired and the logistics of implementing the method, various methods of assessment have been recommended for ear rot assessment. Initial assessment of germplasm can be undertaken by rating the overall ear rot per plot as a whole using a simple logarithmic or linear scale. Accuracy is increased when the percentage infected ears is determined. The ideal and most practical ear rot assessment method for field workers is to determine the percentage infected grain, especially as this is the method used to grade grain when it is delivered to the silos after harvest (Nowell, 1989 and 1995; Nowell and Kaiser, 1989).

Due to the non-linear response of maize hybrids to ear rot under a variety of levels of inoculum pressure, ear rot data should be presented as a response pattern i.e. as the percentage of the mean of the trial or the percentage time a hybrid did better or worse than the mean of the trial. It has been suggested that this could be done using frequency tables (Nowell, 1989, 1992 and 1995; Nowell and Kaiser, 1989). These methods are discussed in detail in Chapters 3, 4 and 6. Flett and McLaren (1994) developed a non-linear regression model that was used to predict hybrid response to ear rot accurately. This model is highly effective in categorising hybrids into susceptible, intermediate and resistant groups. The model can also be used to predict hybrid response to a specific inoculum level. This model showed that hybrids should be screened when disease levels for a trial are between 10 - 35% infected ears (ideally between 17 - 20%). In trials with means on either side of this range, it becomes difficult to accurately differentiate ear rot resistance levels between genotypes.

1.7 Other Factors

There are many environmental and agronomic factors that influence ear rot (Koehler, 1959; Shurtleff, 1980; Palti, 1981; Ferreira, 1994).

1.7.1 Stress

There are many types of stress (e.g., moisture, leaf diseases, hail, cloudy weather, high plant densities and level of fertility) that affect the incidence of ear rots (Koehler, 1959; Pappelis *et al.*, 1973; Dodd, 1980a and 1980b; Berry and Mallet, 1992). Due to the climate in the RSA, drought and temperature stresses are the more common forms of stress. Holbert *et al.* (1935) reported that exposure to chilling and freezing increases both stalk and ear rot susceptibility. Koehler (1959) noted that a period of moisture stress before or during flowering, followed by a relatively moist period, is conducive to ear rots. This finding was supported by Berry and Mallet (1992).

1.7.2 Soil Fertility

There have been a number of publications on the effects of plant and soil nutrition on stalk rots of maize but little has been published on the effects of fertility on ear rots (Koehler, 1959; Kerr, 1965; Farina *et al.*, 1976). While discussing the elements individually, it must be borne in mind that in the field it is an interaction of elements that determines the nutrient balance of the plants. Furthermore, it must also be realised that there are large differences between genotypes in their ability to take up the various nutrients (Otto and Everett, 1956; Martens and Arny, 1967; Porter *et al.*, 1981; Farina *et al.*, 1983).

In South Africa, Farina *et al.* (1976) found that the application of limestone ammonium sulphate significantly increased the incidence of ear rots (predominantly those caused by *S. maydis*, but also by *F. moniliforme* and *F. graminearum*) by between 40 - 100 %. The incidence of ear rot was shown to decrease with a decrease in the level of exchangeable soil acidity.

Koehler (1959) found that the application of additional phosphate to the soil resulted in a

reduction in the incidence of ear rots. This work was supported by Farina *et al.* (1976), who found that the degree of response depends on the initial levels of soil phosphate. Once the phosphate levels increased above 10 ppm, there did not appear to be a further significant reduction in ear rots. It was suggested that phosphate stress, at levels less than 10 ppm soil-tested phosphate, is responsible for the increased ear rot incidence.

Potassium is known to generally increase plant resistance to fungal pathogens. When looking at potassium response over crops and diseases in general, there are more positive effects than negative effects. Literature could not be found that showed a potassium effect on the incidence and/or severity of maize ear rot. The incidence of ear rot was not affected by soil potassium or by negating the effect of nitrogen (Farina *et al.*, 1976).

Kerr (1965) showed that increased nitrogen levels (0 - 30 kg ha^{-1}) decreased the severity of *S. maydis* ear rot in Zimbabwe. However, increased nitrogen resulted in more African stalkborer damage (caused by *Busseola fusca* Fuller), which increased the incidence of *F. moniliforme*. Farina *et al.* (1976) found that the ear rot incidence increased significantly with the incremental application of nitrate nitrogen at levels from 0 - 180 kg ha^{-1}. This effect was consistent over three sites. They suggested the use of nitrification inhibitors as a means of reducing the effect of nitrogen in increasing ear rot incidence.

1.7.3 Plant Architecture

Koehler (1950 and 1959) found that plant architecture was important in reducing ear rots. There was a relationship between ear declination and the prevalence of ear rot, with maize ears that were hanging, rather than erect in relation to the plant, having significantly less ear rot than did the erect ears. Closely associated with the effect of ear declination on ear rots, was the protection of the ear by the husks. Those ears which had loose husks and/or that were open at the tips had significantly more ear rot than the well covered ears, especially in association with the upright ears. Ears of which husks were opened by hand were found to have increased ear rot, and the earlier the husks were opened, the greater the incidence of ear rot. Usually the increase in ear rot was associated with the fusarial pathogens, but in some cases, *S. maydis* increased significantly. Not all hybrids responded as outlined above. A recent study in South Africa showed that ear declination and prolificacy (number of ears per

plant) were correlated to ear rot resistance under South African conditions (Ferreira, 1994).

Lodging of maize plants is influenced by stalk rots which can result in a significantly higher incidence of ear rots. Lodged plants, the ears of which touched the ground, were found not to have increased *S. maydis* ear rot, but there was an increase in ear rot caused by a variety of *Fusarium* species (Koehler, 1959).

1.7.4 Insects

Insects can cause considerable damage to maize plants and are potentially pathogen vectors or cause damage resulting in stress that could predispose the plants to infection by the root, stalk and ear-rotting fungi. Almost all researchers are in agreement that European cornborer (*Ostrinia nubilalis* Hüber) damage results in increased ear rot, primarily caused by *F. moniliforme* and extremely seldom, if ever, caused by *S. maydis* (Koehler, 1959; Kerr, 1965; Jarvis *et al.*, 1982; Keller *et al.*, 1986). *B. fusca* does not increase *S. maydis* ear rot incidence or severity in South Africa (Flett and van Rensburg, 1992). Field observation by the current author, over the past 18 years, showed a good correlation between *B. fusca* and *Chilo partellus* Swinhoe ear infestation and *Fusarium* ear rot only. *S. maydis* ear rot plays no role in stalkborer infestations.

1.7.5 General

General fungicide seed treatments can be used to reduce seedling blights. Captan is the most widely used maize seed fungicide in the world, but a number of other fungicides can also control the seed-borne fungi that are involved in the stalk and ear rot complex (Raju and Lal, 1978; Jain *et al.*, 1981; Pedersen *et al.*, 1986). Raju and Lal (1978) and Pedersen *et al.* (1986) showed that captan is still one of the better broad-spectrum fungicides available. Pedersen *et al.* (1986) claimed that not all hybrids will have the same benefits from the use of a fungicidal seed treatment, and that the main benefits are apparent when maize is planted early, while the soil temperatures are still relatively low, and seedling blight incidence is high.

A potential control measure of seedling blight is biological control. Kommedahl and Mew (1975) found that by applying *Bacillus subtilis* (Cohn) Prazmowski, *Chaetomium globosum*

Kunze ex. Fr. and Captan as a seed treatment, they were able to improve seedling emergence and yield, and reduce lodging and stalk breakage, primarily caused by *G. zeae*. *B. subtilis* had the weakest and most variable response, but the *C. globosum* treatment was comparable to Captan in every respect. They concluded that biocontrol through seed treatment was in its infancy and needed further attention to evaluate its economic feasibility. Kommedahl *et al.* (1987) confirmed the earlier results. *C. globosum* proved to be superior to *B. subtilis* in increasing yield, and to Captan in both grain yield and emergence.

According to Koehler (1959) it is generally accepted that increases in plant density result in an increase in the incidence of ear rot. No literature could be found to substantiate this information. Maize plant density trials conducted during the past 40 years did not measure ear rot incidence or severity.

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CHAPTER 2

Reaction of Maize Hybrids to a Non-invasive *Stenocarpella* Ear Rot Inoculation Technique in KwaZulu-Natal

ABSTRACT

Primary inoculum for maize ear rot epidemics is *Stenocarpella*-contaminated plant debris from the previous season. Ideally, an ear rot inoculation technique would generate inoculum from colonised plant debris, resulting in infection without resorting to artificial physical damage to host plants. In most cases of *Stenocarpella maydis* ear infection, the ear becomes completely rotten and can produce a very significant number of spores the following season, if left in the field. A technique was developed to inoculate maize plants with this colonised material so that an abundance of *Stenocarpella* conidia would be present from the anthesis growth stage onwards. The optimum method of inoculation is to apply 5 - 10 g of finely milled inoculum in the whorl of plants at the 12 - 14 leaf growth stage. This growth stage apparently provides an ideal microclimate for fungal development from this inoculum. Further, the inoculum is ideally placed above the ear, allowing for easy infection of the ear once the stage of ear development and environmental conditions are conducive to infection. This technique was shown to increase ear rot infection consistently. It is ideal for large-scale inoculation as inoculum can be collected and stored easily, and large numbers of plants can be inoculated in a short period with minimal labour requirements. Further, no physical damage to the maize plants occurs.

2.1 INTRODUCTION

Ullstrup (1949) was the first to inoculate maize (*Zea mays* L.) ears in significant numbers with *Stenocarpella maydis* (Berk.) Sutton, to screen for ear rot resistance. He sprayed the ears with conidial and mycelial fragments, and then covered the ears to avoid desiccation of the spores and mycelium. Subsequent to this, a number of researchers have tested a variety of ear rot inoculation methods (Hooker, 1956; Koehler, 1959; Kerr, 1965; Villena, 1969; Ullstrup, 1970 and 1971; Thompson *et al.*, 1971; Chambers, 1988; Rheeder, 1988). However, as early as 1965, Kerr found that introducing mycelium under the ear leaf sheath or physically injecting conidia into the tips of the ears did not give consistent results over seasons, and hybrid ear rot

responses after inoculation were at times poorly correlated with their field responses to ear rot. Warren and Onken (1981) and Warren and von Qualen (1984) developed a technique of introducing *S. maydis* and / or *S. macrospora* Sutton as a spore suspension into the whorl of maize plants 10 - 21 days before anthesis. This produced consistent ear rot expression and results which were consistent with field reactions. Their inoculation technique relied on a natural infection process under suitable environmental conditions, with no wounding.

During 1981, plant breeders at PANNAR (Pty.) Ltd. identified maize ear rot resistance as needing improvement in commercial maize hybrids in South Africa. To achieve this, an effective inoculation technique was needed for use under local conditions. The technique had to be reliable, quick, easy to use and practical. A series of experiments was therefore initiated with the objective of developing an ear rot inoculation method, based on the introduction of infected plant material onto the plants, that could be practically and efficiently employed on a large scale.

2.2 MATERIALS AND METHODS

These trials were planted at Greytown, KwaZulu-Natal, at 29°02'S and 30°31'E at an elevation of 1100 m above sea level and on a gently sloping land of the Hutton form and Doveton series (MacVicar, 1991). The land was prepared by ploughing the fields in September and then discing immediately before planting, to allow for the incorporation of the herbicide Eptam (6 ℓ ha^{-1}). Nitrogen at 110 kg ha^{-1} was incorporated during this process. Rows were made by the planter while band-placing 2:3:2(32)+Zn fertilizer at the rates recommended by the Cedara Agricultural Development Institute (CADI) for a maize grain crop of 8 tonnes ha^{-1}. The maize was hand planted with two kernels per planting hill and then thinned by hand at the five leaf growth stage (LGS) to the correct plant density. Plot size was two rows of 4.4 m long and 0.9 m apart, at a plant density of 50 000 plants ha^{-1}. Plots were separated by a single border row. The complete plot was harvested at the end of the season.

Inoculation was undertaken using milled *Stenocarpella*-colonised ears collected during the previous season. A tractor-powered hammermill was used to mill the colonised ears to the required texture, approximately two months before inoculation took place. Storage of the inoculum was at room temperature in 50 kg bags. Laboratory tests had shown that more than 99% of the *Stenocarpella*-infected ears used for inoculation were co-colonised by *S. maydis* and the balance by *S. macrospora*.

2.2.1 1988/89 Experiments

Two trials were planted in the 1988/89 season. Each trial was planted as a randomised complete block design, with seven inoculation methods replicated four times on the hybrid PNF 6552. The early trial was planted on 7 October 1988 and harvested on 14 May 1989. The late trial was planted on 18 November 1988 and harvested on 21 May 1989. The percentage lodging, prolificacy, the percentage plants that died prematurely, the percentage rotten ears with greater than 10% diseased grain and the percentage prematurely rotten first and second ears were determined before harvest. All assessments were undertaken on an individual plant basis. Grain yield was adjusted to 12.5% grain moisture. Diseased grain was determined by shelling the whole plot, drawing a representative 250 g sample and determining the percentage visually diseased grain based on mass. Only those factors that showed significant differences are presented.

To produce a fine inoculum, the colonised ears were milled into a fine meal, whereas for a coarse inoculum the ears were milled to particles of approximately 2 - 4 mm in diameter. The basic rate of inoculum applied was approximately 2 g plant^{-1}. It was applied into the whorl of the plant approximately 14 days before anthesis, using a commercial coffee dispenser (Nestlé [SA] [Pty] Ltd, Durban, South Africa). The rate of 2N (twice the normal rate of 2 g plant^{-1}) was approximately 4 g plant^{-1} and the 3N (three times the normal rate of 2 g plant^{-1}) inoculum was about 6 g inoculum plant^{-1}.

2.2.2 1989/90 Experiments

In 1989 a single factorial experiment was planted on 4 October 1989 and harvested on 6 May 1990. The trial was replicated four times on the hybrid PNF 6552. The trial consisted of four rates of inoculum applied at six different growth stages (8 LGS, 10 LGS, 12 LGS, 14 LGS, 50% anthesis and 50% anthesis plus 2 weeks). The rates of inoculum applied were 0 g, 5 g, 10 g and 20 g plant^{-1}. The inoculum was placed in the whorl of the plant with a commercial coffee dispenser at the required growth stage. The days to physiological maturity (50% dry husks), percentage lodged plants, prolificacy, grain moisture, 100 kernel weight and grain yield were determined. Days to physiological maturity were taken as an indirect measure of the premature death of the plants (in this case, usually as a result of stalk rot). Grain yield was adjusted to 12.5% grain moisture. Diseased grain was determined by shelling the whole plot,

drawing a representative 250 g sample and determining the percentage visually diseased grain based on mass.

These data were analyzed using GENSTAT Version 5.31 (Rothamstead Experiment Station, United Kingdom) and were not transformed because their high co-efficient of variation was expected and the gain in efficiency from transformation was small. Fischer's L.S.D. test of significance was used to test for significant differences between treatment means.

2.3 RESULTS

2.3.1 1988/89 Experiments

Table 2.1 summarises the results of the early-planted trial. The only factors to show significant differences were the percentage prematurely killed plants and the percentage diseased grain. The differences in the percentage prematurely dead plants were only significant at the 10% level of significance. However, the differences between inoculation methods were highly significant (2% level of significance). Although there were large increases in diseased grain between the non-treated control and the 1N Fine and 1N Coarse methods, the differences were not significant. All other treatments resulted in highly significant increases in diseased grain when compared to the non-treated control. These inoculation methods were not significantly different amongst themselves. The most diseased grain resulted from the 3N Coarse method of inoculation. The coefficient of variation was 35.6%, which is high but still acceptable when measuring a disease of this nature.

Table 2.1 : Summary of the early planted *Stenocarpella maydis* ear rot inoculation trial on PNF 6552 in 1988/89

Treatment	RE1	RE2	%RE	%PD	%DisGr	%Moist	Yield
Control	2.5	17.6	12.8	6.3	5.1 a	15.0	4.812
1N Fine	2.1	13.5	14.1	2.2	15.5 ab	15.2	5.374
2N Fine	0.8	17.8	25.7	4.3	21.1 b	14.9	5.046
3N Fine	4.2	16.2	16.1	5.1	18.8 b	15.1	5.299
1N Coarse	2.2	14.5	21.9	2.6	16.6 ab	15.0	4.781
2N Coarse	0.7	16.7	16.6	9.2	18.2 b	15.2	4.642
3N Coarse	1.5	20.3	26.8	6.7	24.3 b	15.0	5.262
Mean	2.0	16.7	19.1	5.2	17.1	15.0	5.031
SE	2.27	6.65	9.41	3.28	6.08	0.60	0.465
F	1.11	0.46	1.43	2.23	3.92	0.37	1.56
P	n.s.	n.s.	n.s.	0.087	0.01	n.s.	n.s.
$LSD_{0.05}$	4.77	14.0	19.8	6.88	12.8	1.25	0.976
%CV	113.3	39.9	49.1	63.3	35.6	4.0	9.2

Key

RE1	=	percentage rotten first ears at 130 days after planting.
RE2	=	percentage rotten second ears at 130 days after planting.
%RE	=	percentage visually infected ears at harvest.
%PD	=	percentage prematurely dead plants.
%DisGr	=	percentage visually diseased grain.
%Moist	=	percentage grain moisture.
Yield	=	grain yield (t ha^{-1})

The following trends were apparent in the early planted experiment:

i) early ear infection was not affected by inoculation (Figures 2.1 and 2.2)

ii) the percentage colonised ears at harvest increased with increased ear rot inoculum concentrations (Figure 2.3)

iii) the percentage plants dying pre-maturely appeared to decrease with the application of the Fine inoculum treatments (Figure 2.4)

iv) a large increase in diseased grain resulted from all inoculum rates and growth stage of inoculation (Figure 2.5)

v) there was clear effect on grain yield (Figure 2.6).

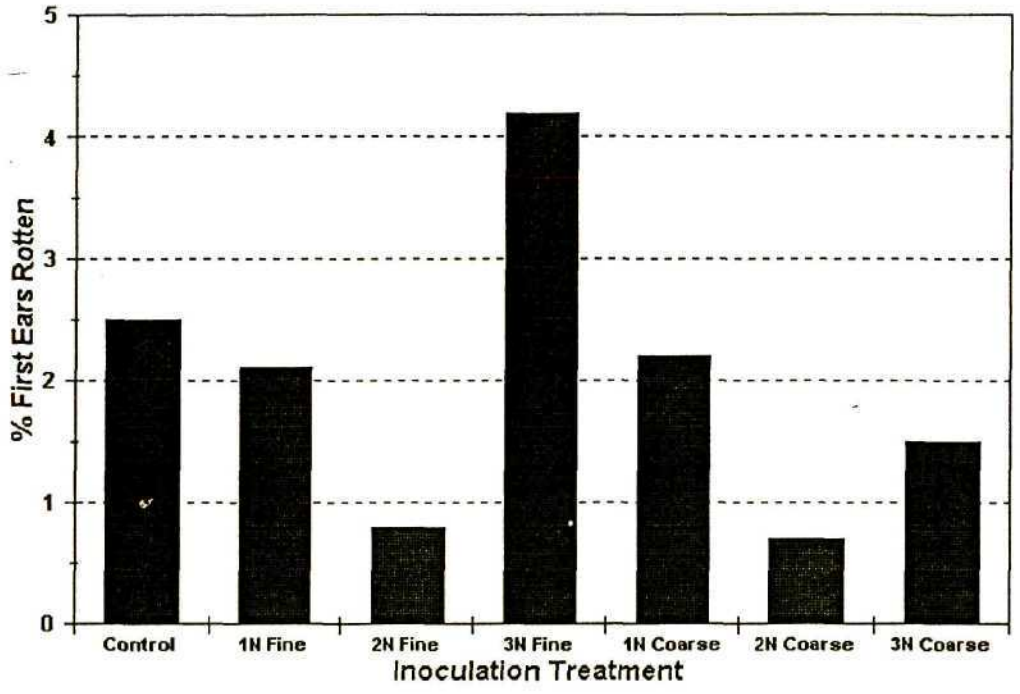

Figure 2.1: The percentage first ears rotten resulting from ear rot inoculations in the whorl of early planted maize in the 1988/89 season.

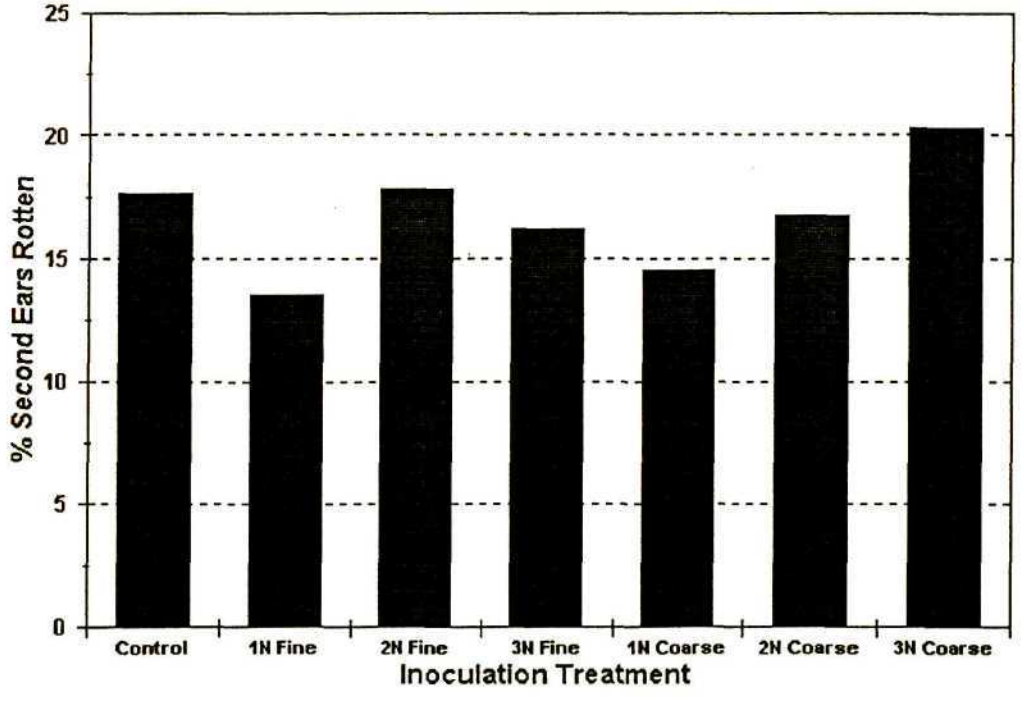

Figure 2.2: The percentage second ears rotten resulting from ear rot inoculations in the whorl of early planted maize in the 1988/89 season.

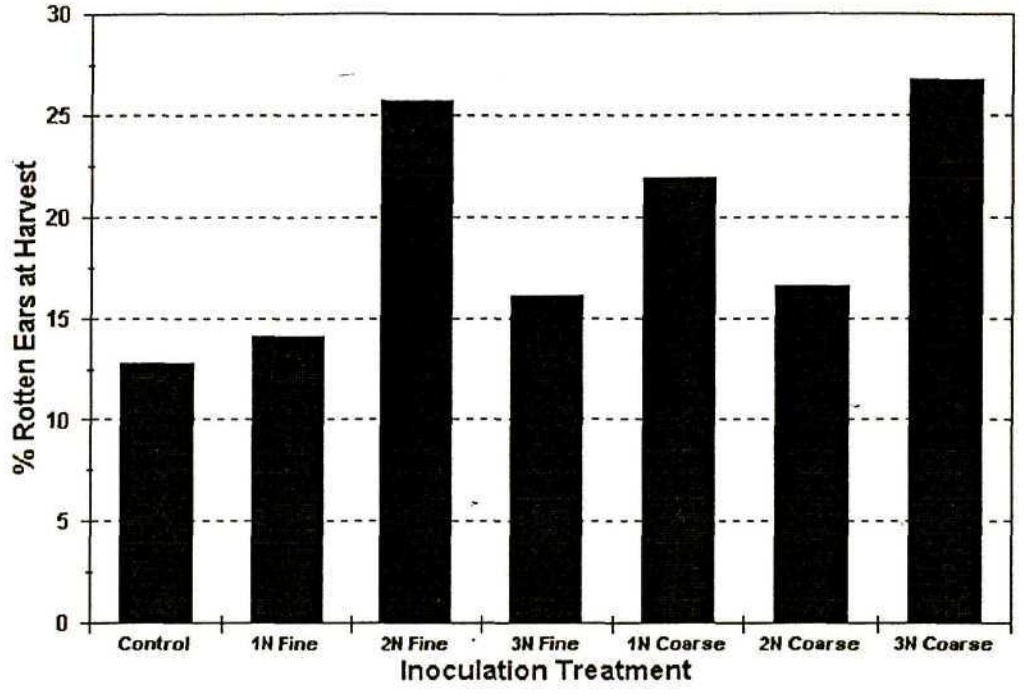

Figure 2.3: The percentage rotten ears at harvest resulting from ear rot inoculations in the whorl of early planted maize in the 1988/89 season.

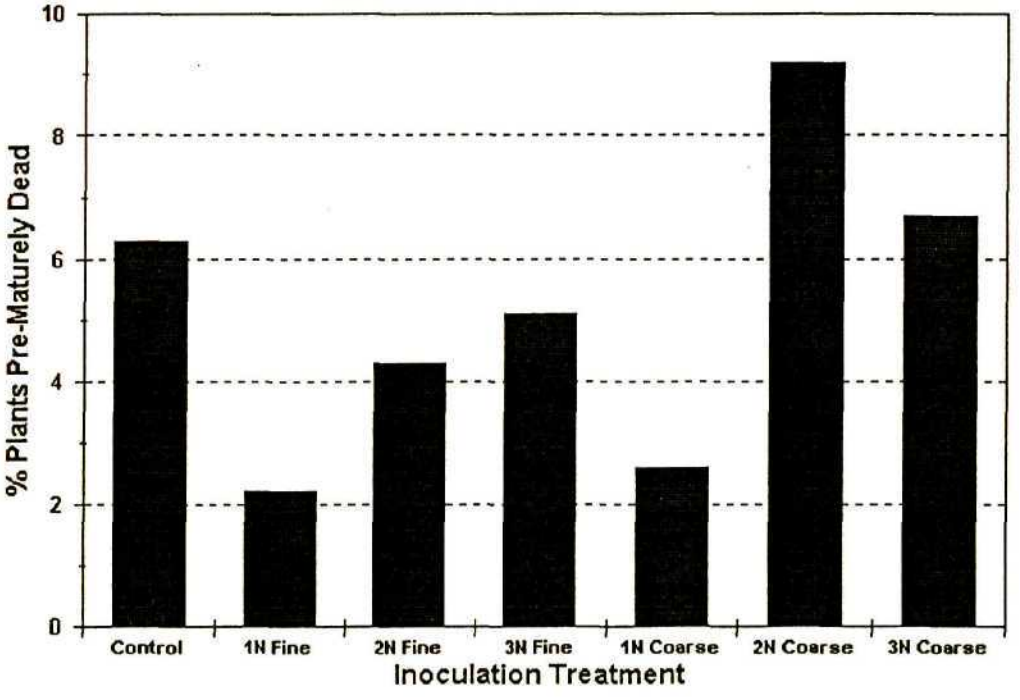

Figure 2.4: The percentage pre-mature dead plants resulting from ear rot inoculations in the whorl of early planted maize in the 1988/89 season.

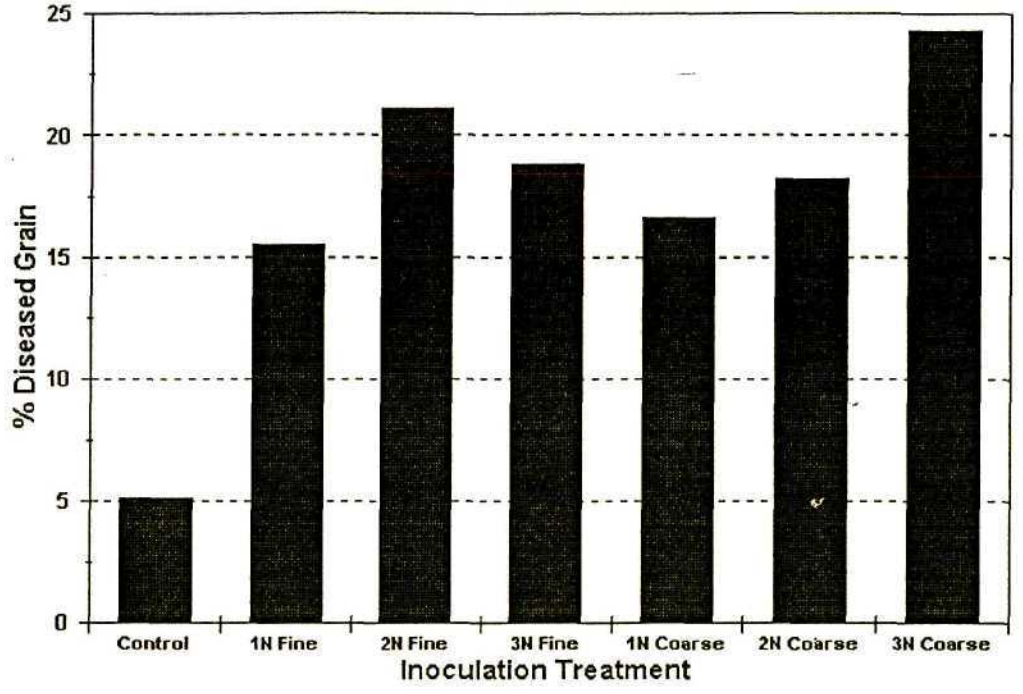

Figure 2.5: The percentage diseased grain resulting from ear rot inoculations in the whorl of early planted maize in the 1988/89 season.

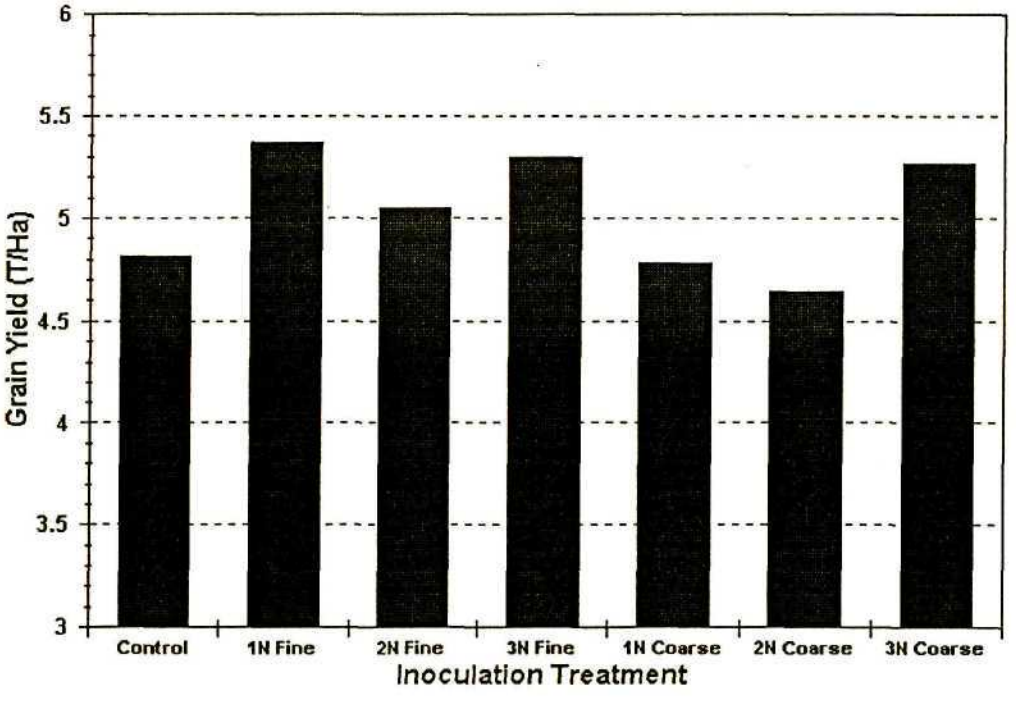

Figure 2.6: The grain yield after ear rot inoculations in the whorl of early planted maize in the 1988/89 season.

Table 2.2 : Summary of the late planted *Stenocarpella maydis* ear rot inoculation trial on PNF 6552 in 1988/89

Treatment	RE1	RE2	%PD	%DisGr	%Moist	Yield
Control	5.6	3.1	2.8	3.8 a	12.3	4.817
1N Fine	6.2	3.1	5.0	6.0 a	12.2	4.631
2N Fine	9.1	4.4	7.2	8.0 a	12.0	4.480
3N Fine	4.1	1.9	3.4	13.8 b	12.2	4.568
1N Coarse	4.7	3.4	2.2	4.1 a	12.2	4.230
2N Coarse	2.8	2.5	6.6	4.2 a	12.4	5.086
3N Coarse	4.4	3.4	5.3	8.1 a	12.3	4.463
Mean	5.3	3.1	4.6	6.9	12.2	4.611
SE	4.02	2.18	5.51	3.23	0.21	0.415
F	0.99	0.52	0.47	4.79	1.49	1.76
P	n.s.	n.s.	n.s.	0.004**	n.s.	n.s.
$LSD_{0.05}$	8.4	4.6	11.6	6.8	0.4	0.87
%CV	76.3	69.6	118.6	47.0	1.7	9.0

Key

RE1 = percentage rotten first ears at 130 days after planting.
RE2 = percentage rotten second ears at 130 days after planting.
%PD = percentage prematurely dead plants.
%DisGr = percentage visually diseased grain.
%Moist = percentage grain moisture.
Yield = grain yield (t ha^{-1})

Table 2.2 summarises the results from the late-planted trial. Only the differences between treatments for the percentage diseased grain were significantly different (1% level of significance). Treatment 3N Fine resulted in a greater amount of diseased grain than the non-treated control. The following trends were apparent, but not significantly different, from the late planted experiment in 1988/89:

i) the 1N and 2N Fine treatments resulted in increased first ears being rotted, but the Coarse treatments all reduced the number of first ears diseased when compared to the non-treated control (Figure 2.7)

ii) no trend emerged with the number of diseased second ears (Figure 2.8)

iii) *S. maydis* inoculation resulted in an increase in the number of pre-maturely dead plants (Figure 2.9)

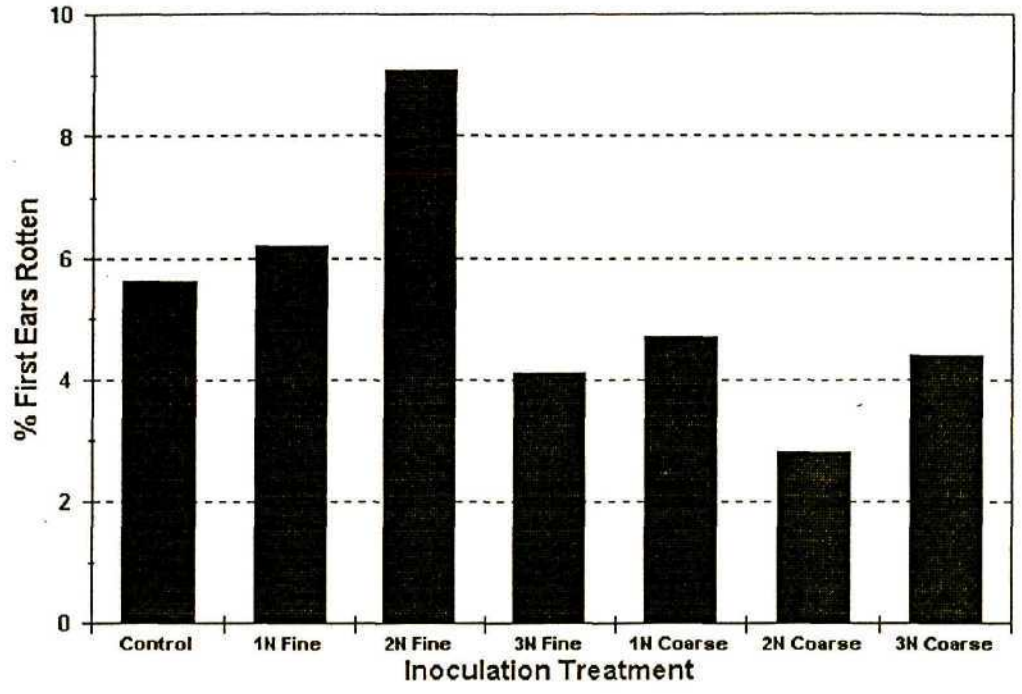

Figure 2.7: The percentage first ears rotten resulting from ear rot inoculations in the whorl of late planted maize in the 1988/89 season.

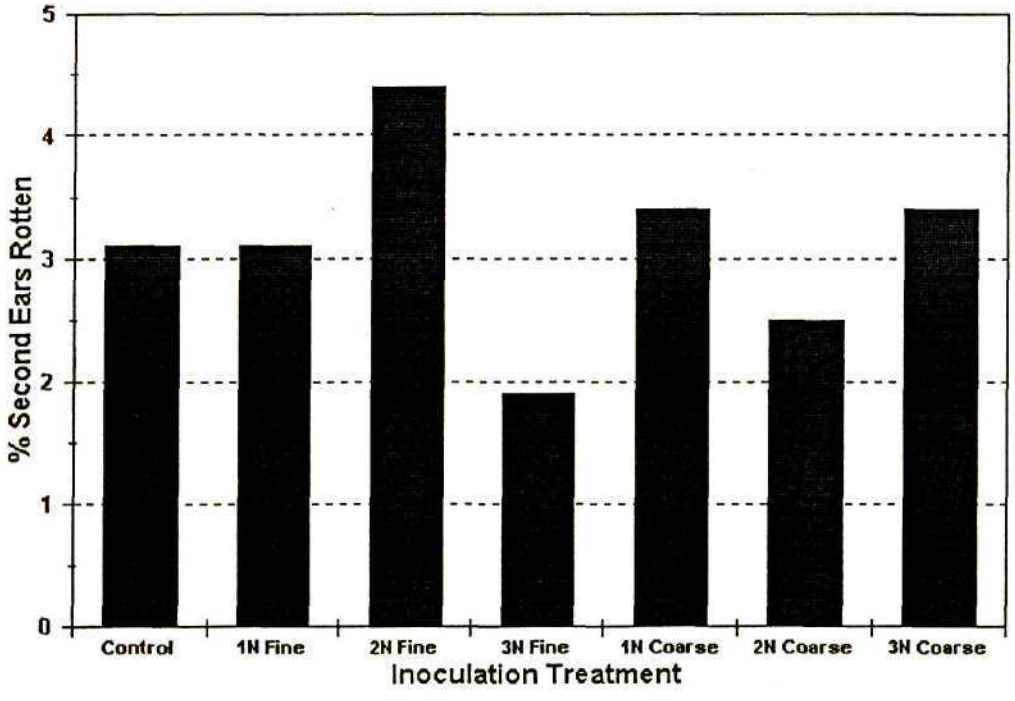

Figure 2.8: The percentage second ears rotten resulting from ear rot inoculations in the whorl of late planted maize in the 1988/89 season.

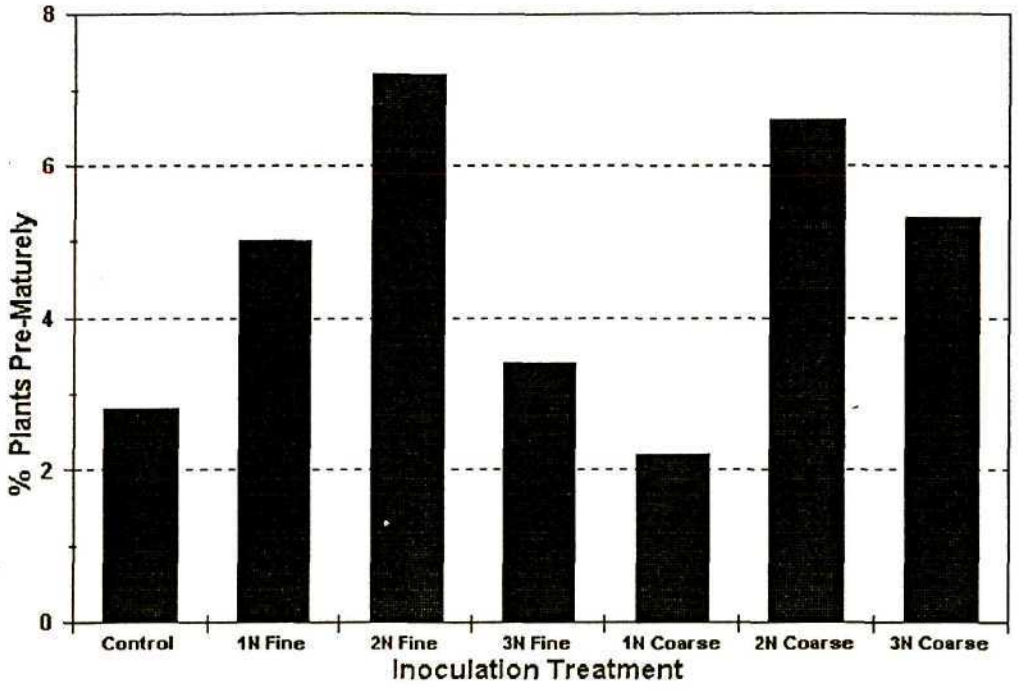

Figure 2.9: The percentage pre-maturely dead plants resulting from ear rot inoculations in the whorl of late planted maize in the 1988/89 season.

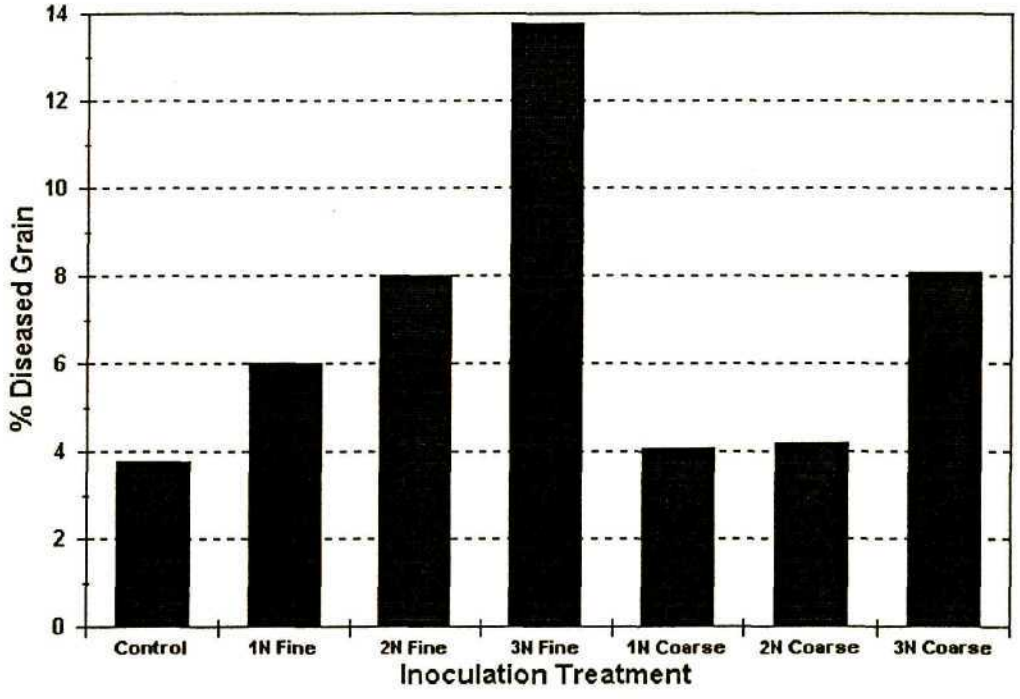

Figure 2.10: The percentage diseased grain at harvest resulting from ear rot inoculations in the whorl of late planted maize in the 1988/89 season.

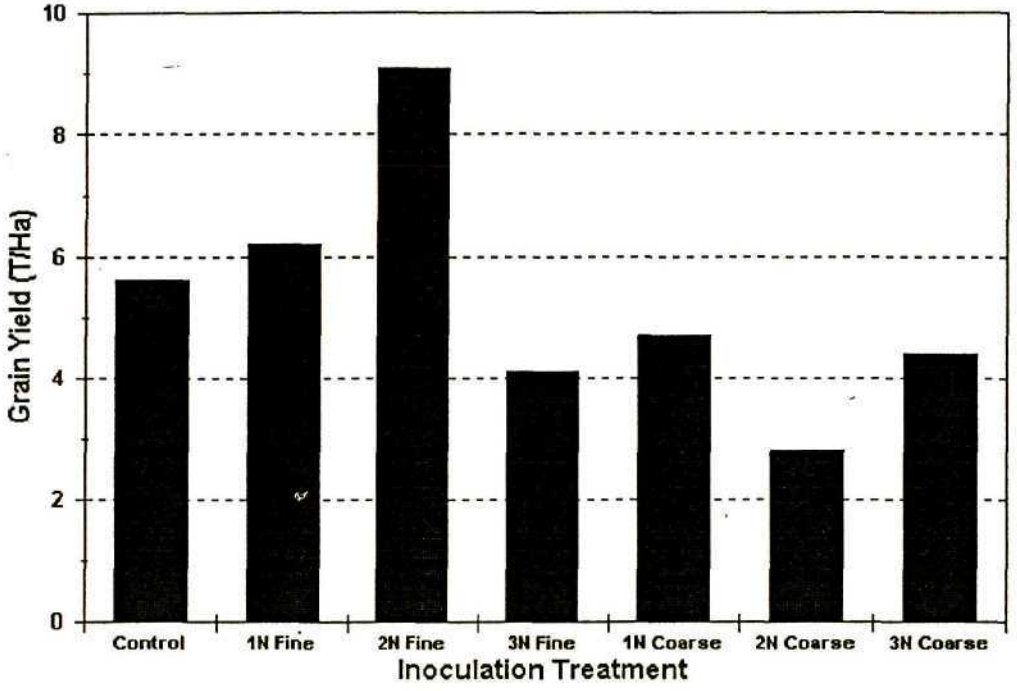

Figure 2.11: The grain yield after ear rot inoculations in the whorl of late planted maize in the 1988/89 season.

iv) the Fine inoculum treatments consistently resulted in increases in the percentage diseased grain. The coarse inoculum treatments only increased the percentage diseased grain at the 3N rate (Figure 2.10)

v) grain yield decreased for all coarse inoculum treatments (Figure 2.11).

2.3.2 1989/90 Experiments

Results are presented in Tables 2.3 - 2.9 and Figure 2.1. The rates of inoculum ($P \leq 0.05$) reduced the number of days to physiological maturity (an indirect measure of stalk rot and / or disease effect on the plant), as shown in Table 2.3 and Figures 2.12 and 2.13. From these data it can be seen that the rate of 10g of inoculum per plant reduced the number of days to maturity significantly, particularly when inoculated at the 10 LGS and 12 LGS. The results of the 10g inoculum rate were not different from the 5g or 20g inoculum rate.

Table 2.3 : Summary of the days to physiological maturity for the ear rot inoculation trial on PNF 6552 in 1989/90

Maize Growth Stage	Rate of Inoculum Applied				
	0	5	10	20	Mean
8 Leaves	146.0	147.7	143.3	146.0	145.8
10 Leaves	149.3	149.7	144.7	147.7	147.8
12 Leaves	147.7	147.7	145.0	141.7	145.5
14 Leaves	146.7	145.0	146.3	145.3	145.8
50% Anthesis	145.0	147.3	145.0	145.0	145.6
50% Anth. & 2 weeks	147.0	143.3	143.7	144.3	144.6
Mean	146.9 a	146.8 ab	144.7 b	145.0 ab	145.8

SUMMARY OF THE ANOVA RESULTS			
Main effects			
	Stage of inoculation	F = 1.63	P = n.s.
	Rate of inoculum	F = 2.06	P = 0.042*
Interaction effects			
	Stage x Rate	F = 0.92	P = n.s.
$LSD_{0.05}$	Stage	3.3	
$LSD_{0.05}$	Rate	2.1	
$LSD_{0.05}$	Stage x Rate	5.1	
% CV		2.0	

Figure 2.12 shows the decrease in days to physiological maturity with increased inoculum pressure. Figure 2.13 shows that the least effect on days to physiological maturity was when maize was inoculated at the 10 LGS.

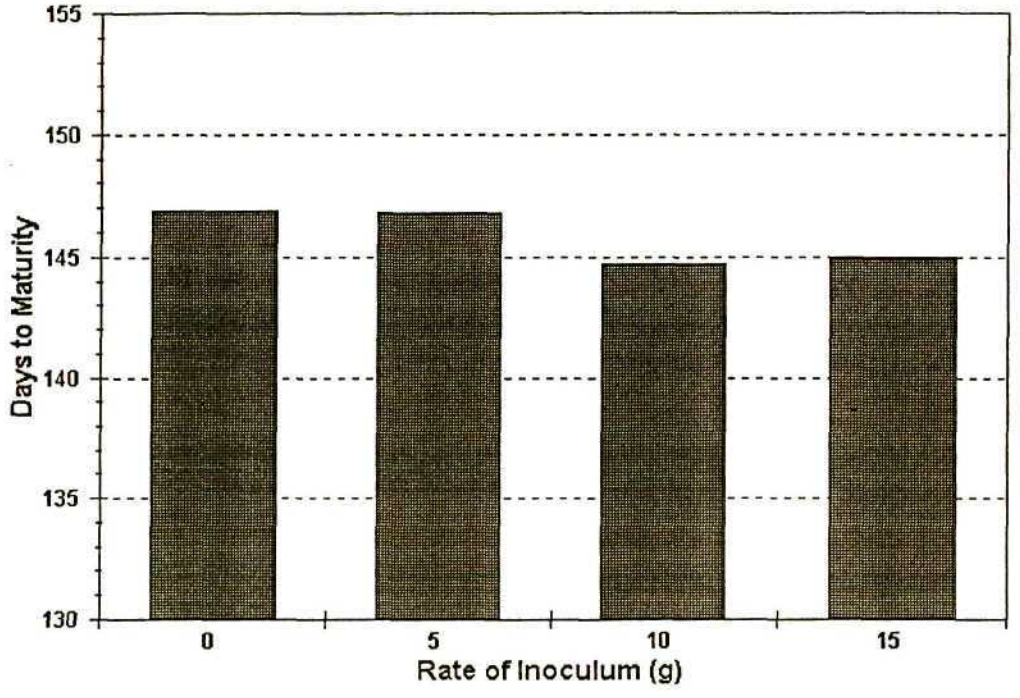

Figure 2.12: The days to physiological maturity after ear rot inoculations in the whorl of the maize plants at different inoculum concentrations in the 1989/90 season.

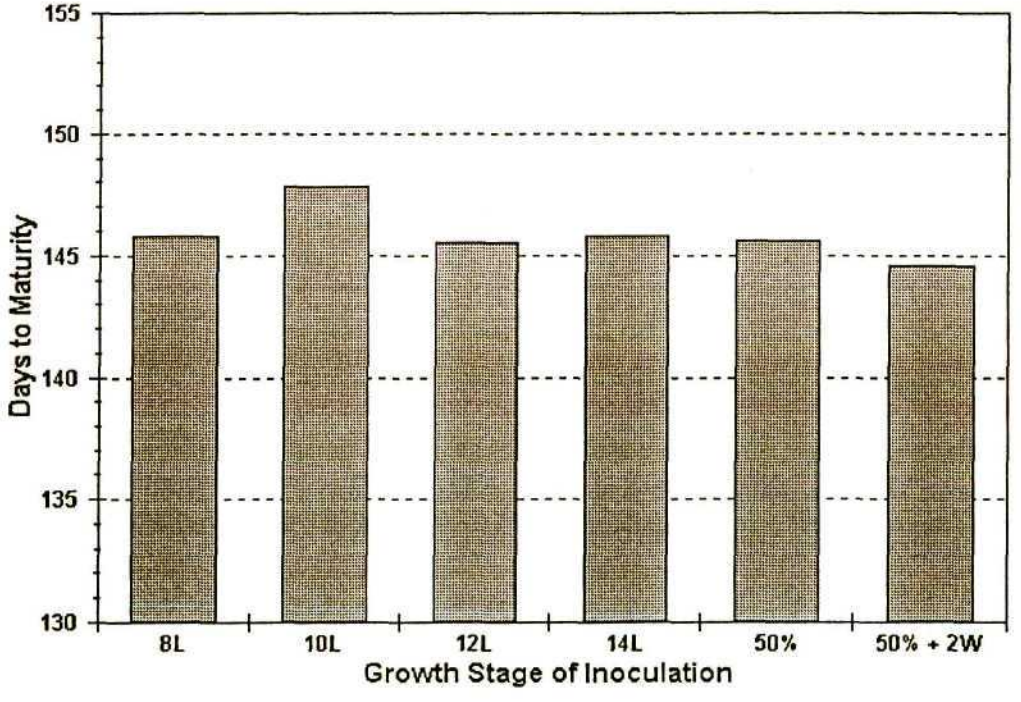

Figure 2.13: The days to maturity after ear rot inoculations in the whorl of the maize plants at different growth stages during the 1989/90 season.

Table 2.4 : Summary of the total lodging for the ear rot inoculation trial on PNF 6552 in 1989/90

Maize Growth Stage	Rate of Inoculum Applied				
	0	5	10	20	Mean
8 Leaves	31.2	15.4	13.4	21.6	20.4
10 Leaves	25.0	20.2	22.1	18.1	21.4
12 Leaves	37.5	21.6	12.3	22.5	23.5
14 Leaves	22.1	36.4	23.7	39.2	30.3
50% Anthesis	38.7	18.4	26.3	19.3	25.7
50% Anth. & 2 weeks	30.6	20.0	23.5	20.5	23.6
Mean	30.8 a	22.0 ab	20.2 b	23.5 ab	24.1

SUMMARY OF THE ANOVA RESULTS

Main effects			
	Stage of inoculation	F = 1.73	P = n.s.
	Rate of inoculum	F = 4.47	P = 0.008**
Interaction effects			
	Stage x Rate	F = 1.63	P = n.s.
$LSD_{0.05}$	Stage	10.2	
$LSD_{0.05}$	Rate	9.8	
$LSD_{0.05}$	Stage x Rate	26.4	
% CV		38.8	

An increase in ear rot inoculum reduced the amount of lodging (Table 2.4 and Figure 2.14). The percentage lodging was reduced by the 10 g inoculum rate when compared to the non-treated control. The percentage lodged plants resulting from the different rates of inoculum were not different from each other. Figure 2.15 shows that inoculation reduced the percentage lodged plants compared to the non-treated control.

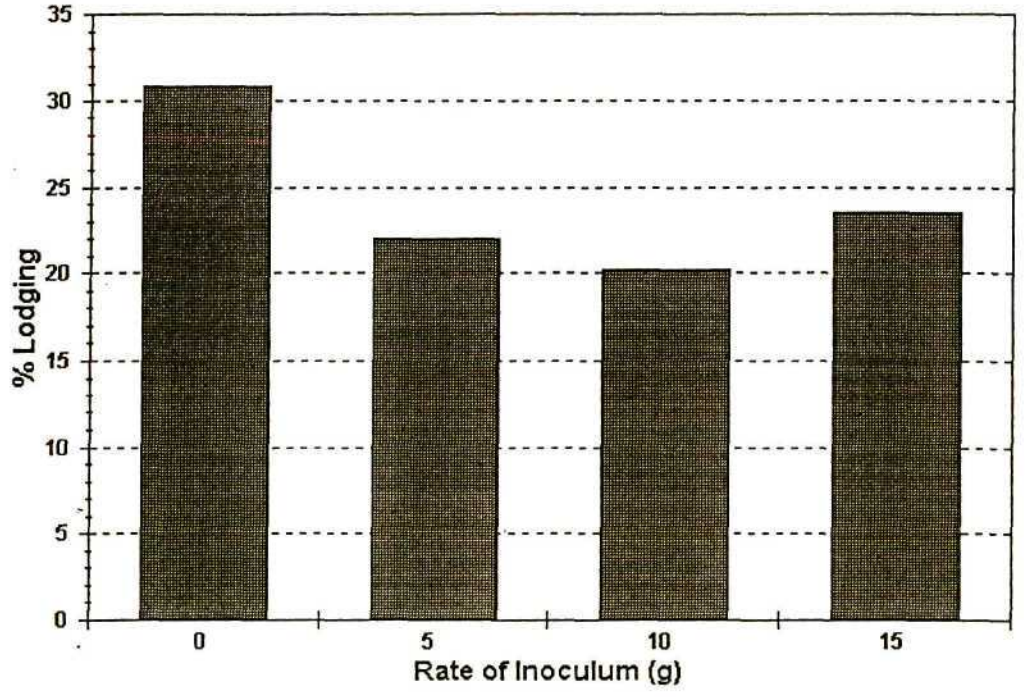

Figure 2.14: The percentage lodged plants after ear rot inoculations in the whorl of the maize plants at different inoculum concentrations during the 1989/90 season.

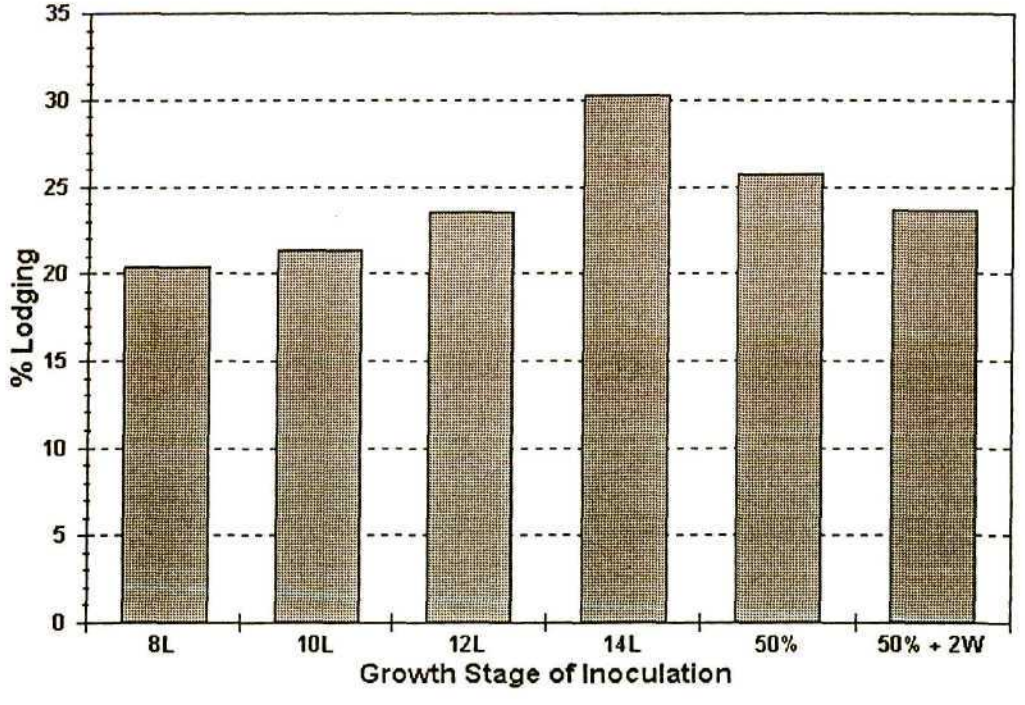

Figure 2.15: The percentage lodged plants after ear rot inoculations in the whorl of the maize plants at different growth stages during the 1989/90 season.

Table 2.5 : Summary of the prolificacy for the ear rot inoculation trial on PNF 6552 in 1989/90

Maize Growth Stage	Rate of Inoculum Applied (g plant^{-1})				
	0	5	10	20	Mean
8 Leaves	0.974	0.959	0.935	1.029	0.974 a
10 Leaves	0.893	0.936	0.929	0.964	0.930 ab
12 Leaves	0.960	1.022	0.958	0.983	0.981 a
14 Leaves	0.908	0.950	0.905	0.976	0.935 ab
50% Anthesis	0.887	0.878	0.924	0.873	0.891 b
50% Anth. & 2 weeks	0.949	0.942	0.983	0.943	0.954 a
Mean	0.928	0.948	0.939	0.961	0.944

SUMMARY OF THE ANOVA RESULTS			
Main effects			
	Stage of inoculation	F = 2.73	P = 0.031*
	Rate of inoculum	F = 0.71	P = n.s.
Interaction effects			
	Stage x Rate	F = 0.53	P = n.s.
$LSD_{0.05}$	Stage	0.060	
$LSD_{0.05}$	Rate	0.049	
$LSD_{0.05}$	Stage x Rate	0.161	
% CV		7.4	

Prolificacy was not affected by the rate of inoculum applied but was influenced by the growth stage at which the inoculum was applied (see Table 2.5 and Figure 2.16). When inoculum was applied at 50% anthesis, prolificacy was reduced when compared to the non-treated control. All other treatments did not differ from each other, although the trend was for reduction in prolificacy (Figure 2.17).

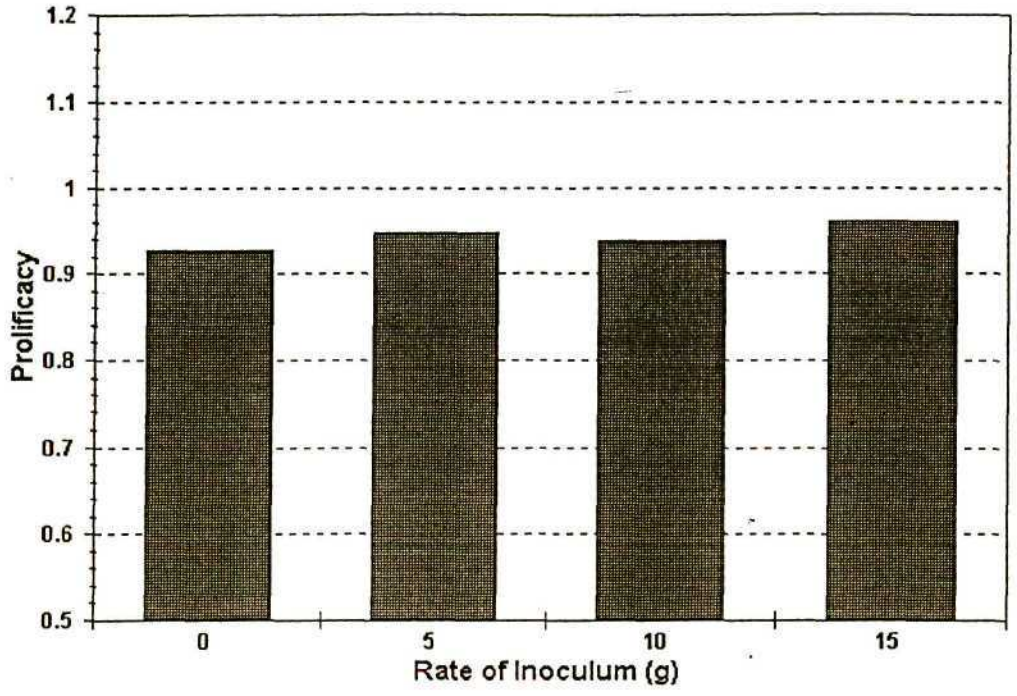

Figure 2.16: Prolificacy after ear rot inoculations in the whorl of the maize plants at different inoculum concentrations during the 1989/90 season.

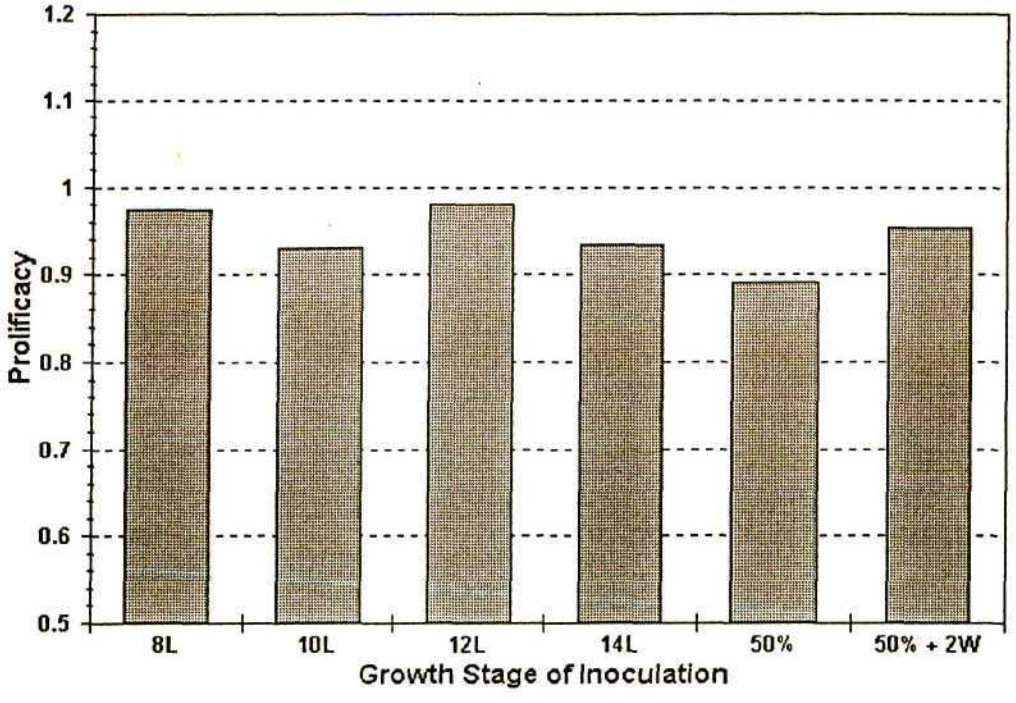

Figure 2.17: Prolificacy after ear rot inoculations in the whorl of the maize plants at different growth stages during the 1989/90 season.

Table 2.6 : Summary of the percentage diseased grain for the ear rot inoculation trial on PNF 6552 in 1989/90

Maize Growth Stage	Rate of Inoculum Applied (g plant^{-1})				
	0	5	10	20	Mean
8 Leaves	9.80	15.57	11.30	13.63	12.58
10 Leaves	11.07	11.97	11.17	18.37	13.14
12 Leaves	12.20	19.33	21.57	21.77	18.72
14 Leaves	12.57	17.00	19.87	17.30	16.68
50% Anthesis	11.90	14.70	16.37	21.53	16.12
50% Anth. & 2 weeks	11.13	15.90	15.23	22.67	16.23
Mean	11.44 a	15.74 b	15.92 b	19.21 b	15.20

SUMMARY OF THE ANOVA RESULTS

Main effects			
	Stage of inoculation	F = 1.07	P = n.s.
	Rate of inoculum	F = 5.08	P = 0.004**
Interaction effects			
	Stage x Rate	F = 1.73	P = 0.079
$LSD_{0.05}$	Stage	6.89	
$LSD_{0.05}$	Rate	3.99	
$LSD_{0.05}$	Stage x Rate	9.78	
% CV		37.0	

The percentage diseased grain increased for all rates of inoculum applied (Table 2.6 and Figure 2.18) when compared to the non-treated control. There were no differences between the different rates of inoculum applied. Although the growth stage at inoculation did not result in any significant differences in diseased grain, there was a definite trend for inoculations at the 12 LGS to result in more ear rot, especially at the 12 LGS (Figure 2.19). The 10 g or 20 g inoculum rate resulted in significantly more diseased grain when inoculation took place at the 12 LGS than at either the 8 or 10 LGS. The non-treated control at the 0 g inoculum rate, had an unexpectedly low percentage diseased grain.

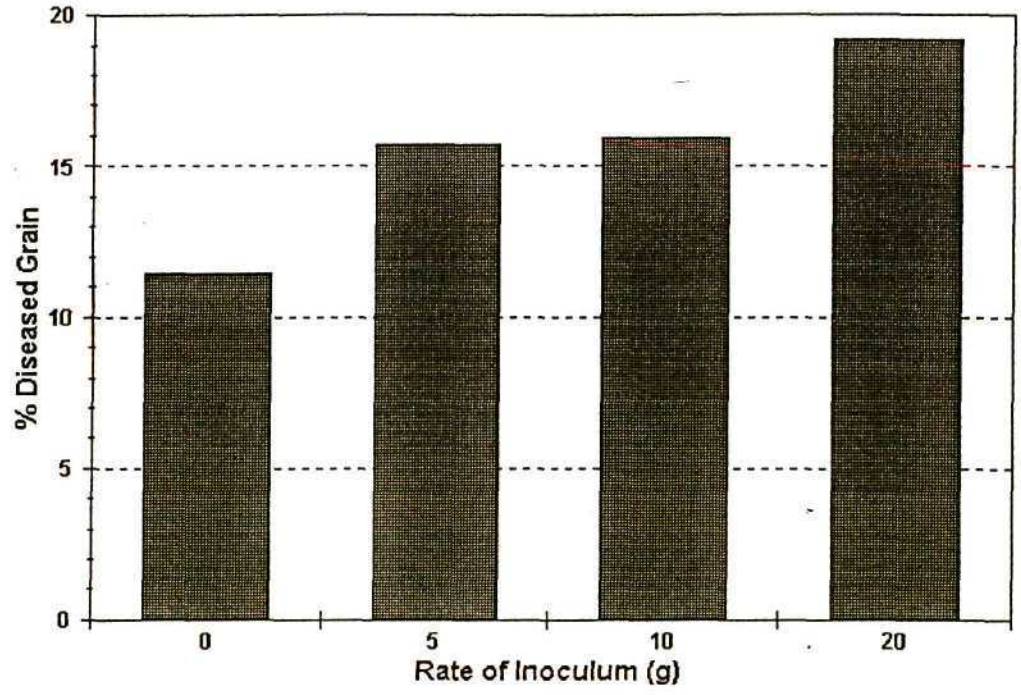

Figure 2.18: The percentage diseased grain after ear rot inoculations in the whorl of the maize plants at different inoculum concentrations during the 1989/90 season.

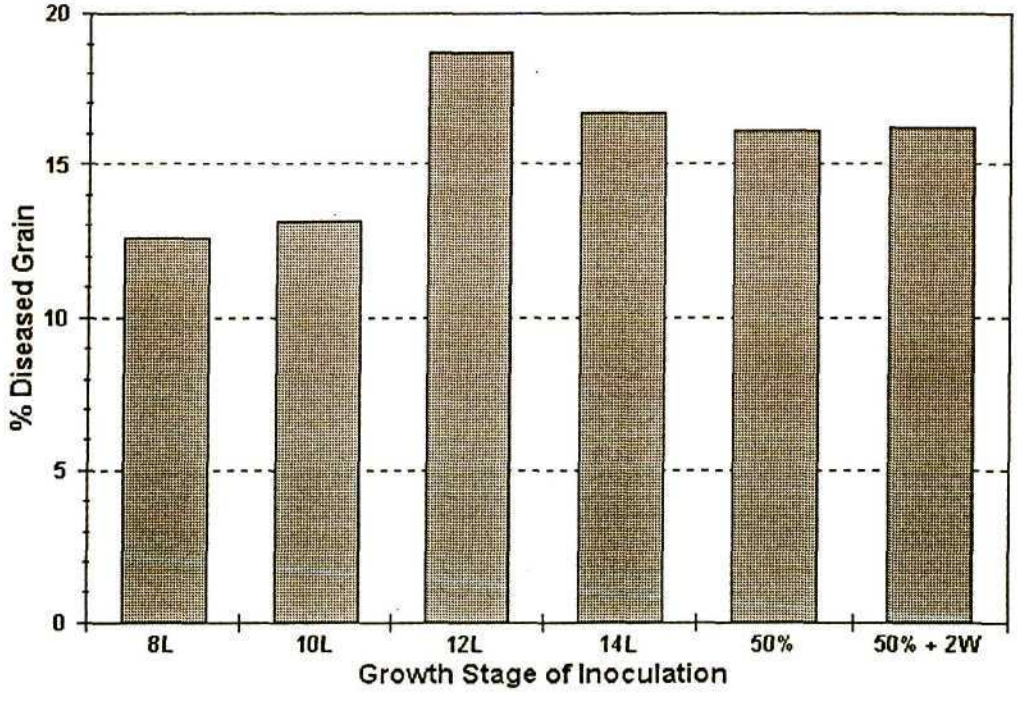

Figure 2.19: The percentage diseased grain after ear rot inoculations in the whorl of the maize plants at different growth stages during the 1989/90 season.

Table 2.7 : Summary of the percentage grain moisture for the ear rot inoculation trial on PNF 6552 in 1989/90

Maize Growth Stage	Rate of Inoculum Applied (g plant^{-1})				
	0	5	10	20	Mean
8 Leaves	12.40	12.77	12.17	12.67	12.50 ab
10 Leaves	12.13	12.43	12.40	12.07	12.26 a
12 Leaves	12.73	12.70	12.80	12.77	12.75 bc
14 Leaves	12.83	13.57	13.37	12.83	13.15 c
50% Anthesis	13.07	12.33	12.40	13.13	12.73 bc
50% Anth. & 2 weeks	12.60	12.67	13.13	13.03	12.86 bc
Mean	12.63	12.74	12.71	12.75	12.71

SUMMARY OF THE ANOVA RESULTS			
Main effects			
	Stage of inoculation	F = 4.58	P = 0.002**
	Rate of inoculum	F = 0.23	P = n.s.
Interaction effects			
	Stage x Rate	F = 1.18	P = n.s.
$LSD_{0.05}$	Stage	0.43	
$LSD_{0.05}$	Rate	0.35	
$LSD_{0.05}$	Stage x Rate	1.56	
% CV		3.9	

The effects of inoculations on grain moisture are presented in Table 2.7 and Figures 2.20 and 2.21. Grain moisture was not significantly influenced by the rate of inoculum applied. Inoculation at the 12 LGS resulted in an increase in grain moisture. Ear rot inoculations from the 10 LGS onwards were not different from each other. The trend was for a decrease in grain moisture at harvest when inoculation took place at the 8 LGS and 10 LGS. Inoculation at the 14 LGS appeared to increase grain moisture at harvest.

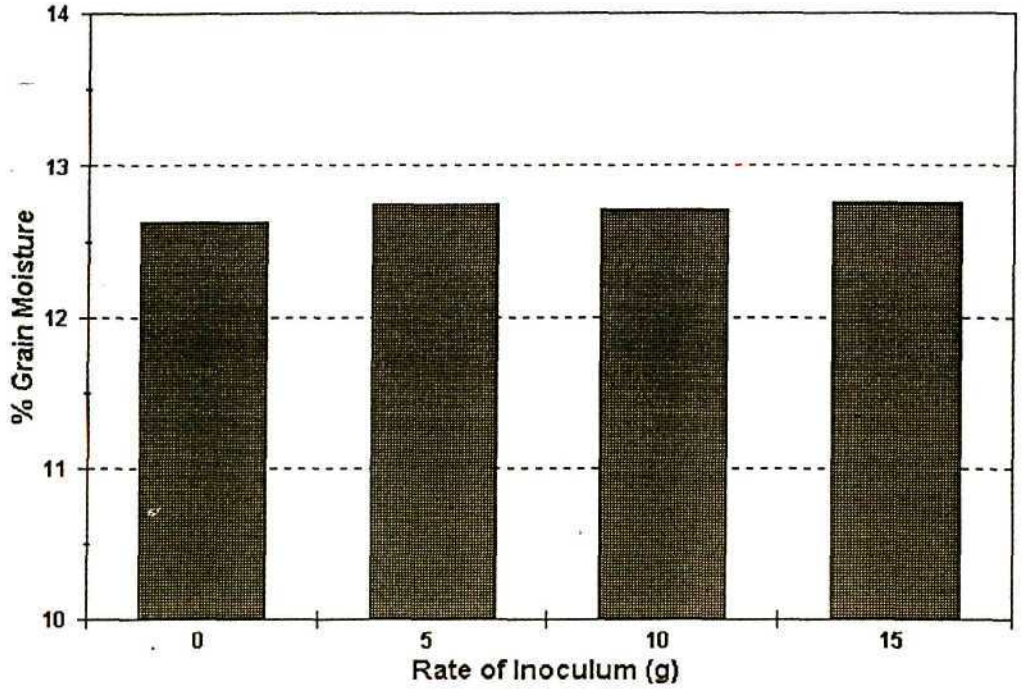

Figure 2.20: The percentage grain moisture after ear rot inoculations in the whorl of the maize plants using different inoculum concentrations during the 1989/90 season.

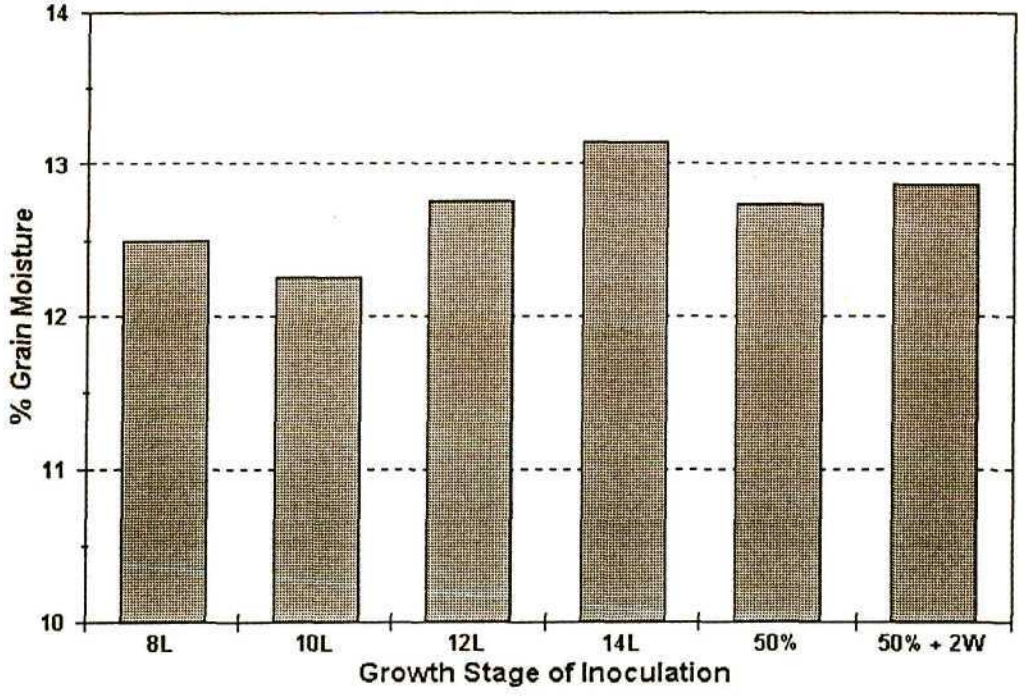

Figure 2.21: The percentage grain moisture after ear rot inoculations in the whorl of the maize plants at different growth stages during the 1989/90 season.

Table 2.8 : Summary of the 100-kernel weight (g) for the ear rot inoculation trial on PNF 6552 in 1989/90

Maize Growth Stage	Rate of Inoculum Applied (g plant^{-1})				
	0	5	10	20	Mean
8 Leaves	37.83	35.37	36.83	35.53	36.39 a
10 Leaves	37.37	36.23	35.80	35.67	36.27 a
12 Leaves	36.90	38.20	39.00	39.00	38.28 c
14 Leaves	36.40	36.50	38.50	38.43	37.46 abc
50% Anthesis	37.07	35.67	36.93	37.93	36.90 ab
50% Anth. & 2 weeks	38.60	37.63	36.87	37.70	37.70 bc
Mean	37.36	36.60	37.32	37.38	37.17

SUMMARY OF THE ANOVA RESULTS

Main effects			
	Stage of inoculation	F = 3.75	P = 0.006**
	Rate of inoculum	F = 1.30	P = n.s.
Interaction effects			
	Stage x Rate	F = 1.46	P = n.s.
$LSD_{0.05}$	Stage	1.22	
$LSD_{0.05}$	Rate	1.00	
$LSD_{0.05}$	Stage x Rate	3.59	
% CV		3.8	

The rate of inoculum applied did not influence the 100-kernel weight (Figure 2.22) but was influenced by the growth stage at which the inoculum was applied (Table 2.8 and Figure 2.23). Inoculation at the 12 LGS and 50% anthesis plus 2 weeks growth stages resulted in increased 100-kernel weight. Results from the inoculation at the 10 LGS, 14 LGS and 50% anthesis were not different from each other. The trend was for an increase in 100-kernel weight with later inoculation.

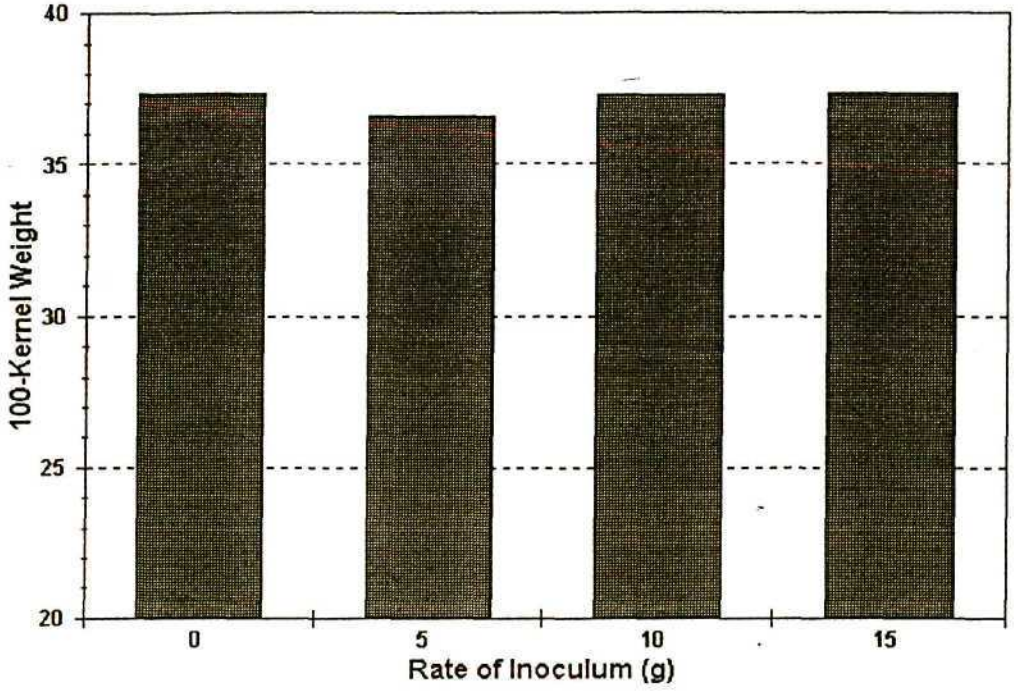

Figure 2.22: The 100-kernel weight after ear rot inoculations in the whorl of the maize plants using different inoculum concentrations during the 1989/90 season.

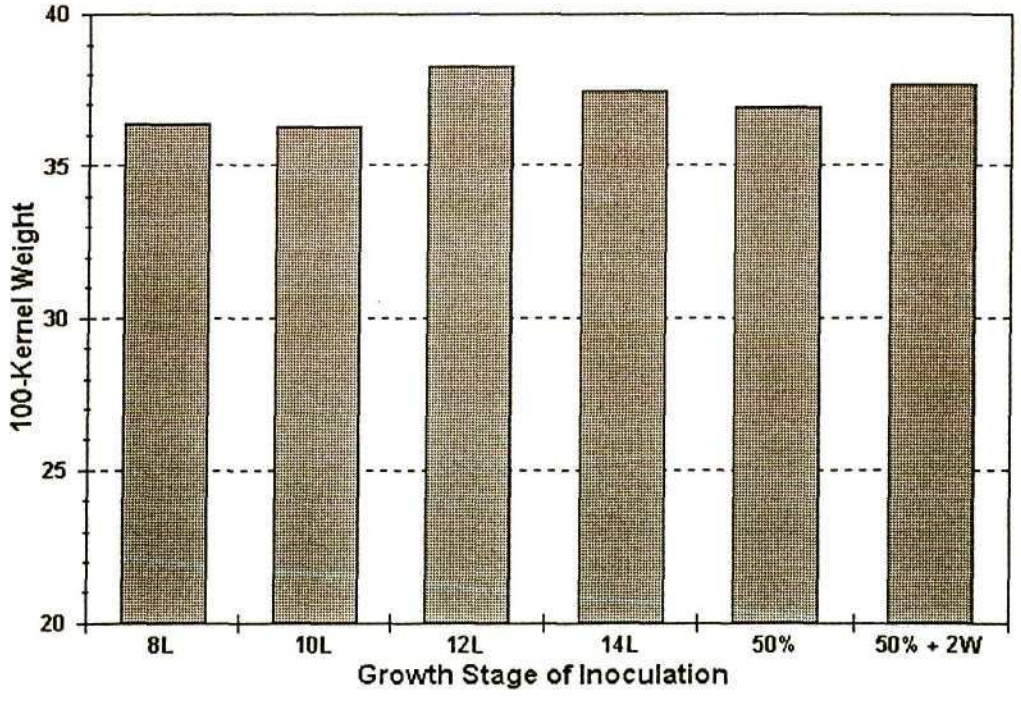

Figure 2.23: The 100-kernel weight after ear rot inoculations in the whorl of the maize plants at different growth stages during the 1989/90 season.

Table 2.9 : Summary of the grain yield (t ha^{-1}) for the ear rot inoculation trial on PNF 6552 in 1989/90

Maize Growth Stage	Rate of Inoculum Applied (g $plant^{-1}$)				
	0	5	10	20	Mean
8 Leaves	6.667	4.933	5.967	5.900	5.867 ab
10 Leaves	6.700	5.967	6.367	5.667	6.175 ab
12 Leaves	6.867	6.233	6.433	5.867	6.350 ab
14 Leaves	6.800	7.067	5.900	6.067	6.458 a
50% Anthesis	6.633	5.167	5.833	5.367	5.750 ab
50% Anth. & 2 weeks	6.500	5.967	5.533	4.733	5.683 b
Mean	6.694 a	5.889 b	6.006 ab	5.600 b	6.047

SUMMARY OF THE ANOVA RESULTS			
Main effects			
	Stage of inoculation	F = 2.56	P = 0.040*
	Rate of inoculum	F =7.78	P = <0.001**
Interaction effects			
	Stage x Rate	F = 1.08	P =n.s.
$LSD_{0.05}$	Stage	0.741	
$LSD_{0.05}$	Rate	0.748	
$LSD_{0.05}$	Stage x Rate	1.228	
% CV		11.7	

The application of *S. maydis* inoculum reduced grain yield (Table 2.9 and Figure 2.24). The growth stage at which inoculum was applied was important. The lowest grain yield resulted from inoculation at the 50% Anthesis plus 2 Weeks growth stage (Figure 2.25). The overall trend was for grain yield to be least affected when inoculation took place at the 14 LGS. Grain yield reductions were largest when inoculation took place from anthesis onwards.

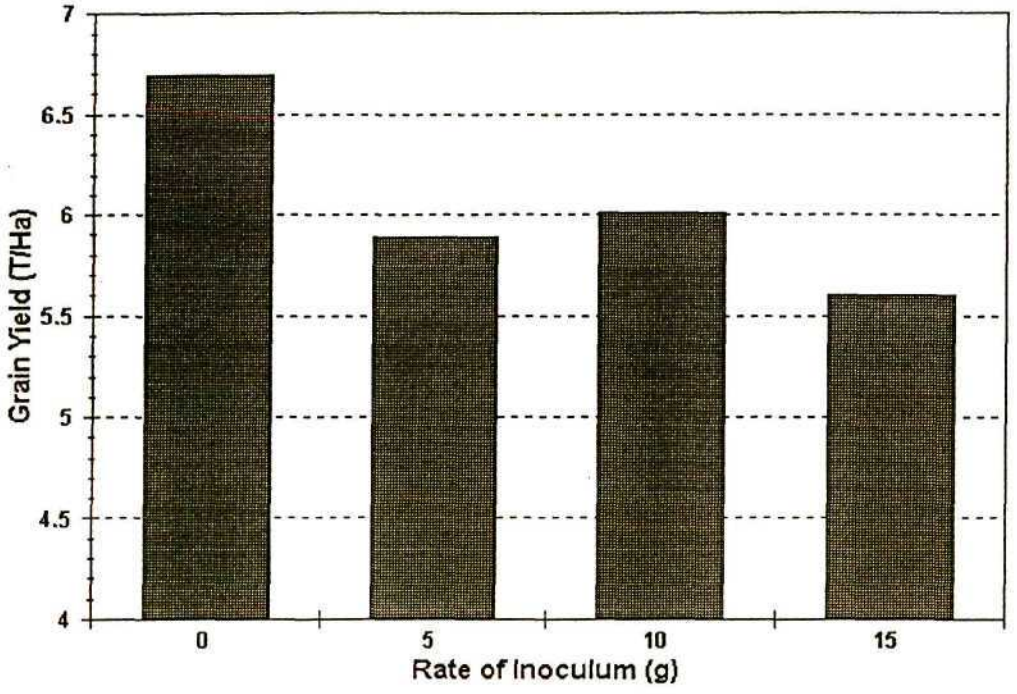

Figure 2.24: The grain yield after ear rot inoculations in the whorl of the maize plants at different inoculum concentrations during the 1989/90 season.

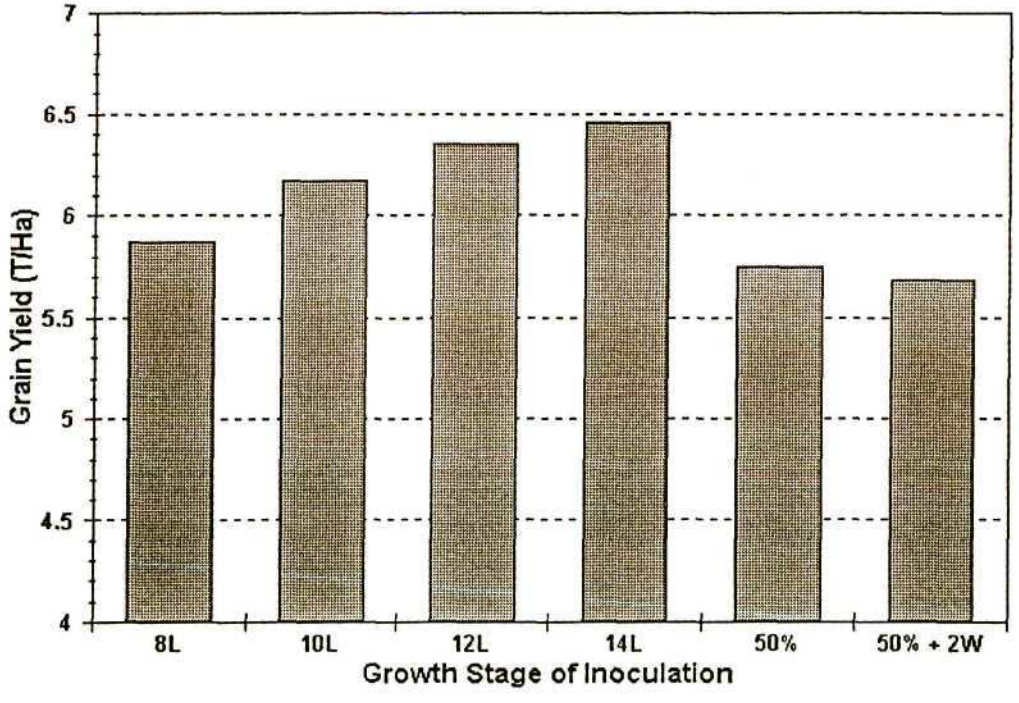

Figure 2.25: The grain yield after ear rot inoculations in the whorl of the maize plants at different growth stages during the 1989/90 season.

2.4 DISCUSSION

The application of milled *Stenocarpella*-infected ears to the whorl of maize plants is highly effective in increasing the incidence and severity of ear rot. The increased levels of ear rot, after the application of ear rot inoculum, were consistent over seasons, although the planting date did influence the severity of ear rot. This was expected as the maize in the late planted experiment, pollinated and matured during a period that was drier and less conducive to ear rot infection and development (Table 2.2). Under conditions less than ideal for ear rot, it is still possible to induce an ear rot epidemic by increasing the amount of inoculum applied plant^{-1}. Ideally, screening for ear rot resistance should take place early in the season when conditions are conducive to ear rot infection and development. The careful use of irrigation would likely increase the effectiveness of inoculating late planted maize and increase ear rot incidence and severity.

The 1988/89 experiments showed that the application of ear rot inoculum reduced the number of days to maturity; i.e., the rate of plant senescence was increased. This increased rate of senescence was supported by a reduction in grain moisture of some treatments and the increase in the percentage premature dead plants. This is likely to be as a result of increased stalk rot, as reported by Warren and von Qualen (1984).

The decrease in the percentage lodged plants associated with the early and late *S. maydis* inoculation growth stages, correlated well with the associated grain yield decreases. The 1989/90 growing season was not ideal for ear rot development or maize production, as the latter half of the season had a reduced rainfall. Optimum maize production conditions would have resulted in larger grain yields and this would have influenced lodging significantly. The magnitude of the effect depended on the environmental conditions under which the trial developed from the time of inoculation. This research needs to be repeated under a number of different climatic conditions in order to determine factors that affect ear rot development. Effects are likely to be larger in more moist seasons than in drier seasons.

The prolificacy of the plants was reduced if inoculation took place during anthesis and silk emergence. The soft tissue of the emerging ears is likely to be the infection site for *Stenocarpella* species. Severe infection would result in the death of the ear. This would lead to a reduction in prolificacy. Climatic conditions during this stage of plant growth were also

ideal for infection to take place. The development of stalk rot after inoculation after these stages of plant development was minimal. Early and late ear rot inoculations resulted in the greatest amount of stalk rot and had the largest affect in reducing yield.

The differences in the visually early diseased first and / or the second ears were not significant between treatments. There was a greater number of diseased second ears than first ears in the early planted experiment in 1988/89. This trend was not apparent in the late planted experiment. This shows that under ideal ear rot conditions, ear rot infection starts on the smaller and less developed second ears.

The rate and type of ear rot inoculum applied generally had more effect on the severity of ear rot, as measured by the percentage visually diseased grain, than the growth stage at which inoculation took place. In 1988/89, there were no differences between the type and rate of inoculum applied on the early experiment. However, the only effective treatment of the late trial was the 3N Fine treatment that was applied. During 1989/90, inoculation after the 12 LGS resulted in an increase in the percentage diseased grain (but differences were not significant).

The percentage grain moisture and 100-kernel weight was significantly affected by the stage at which the plants were inoculated. Overall, 100-kernel weight increased from the 8 LGS to the 12 LGS when the plants were inoculated with ear rot inoculum. However, at these early growth stages, the 100-kernel weight was lower than the non-treated control. This was as a result of the effect of stalk rot on the development of the ears resulting from the prolonged exposure to *Stenocarpella*. Inoculation from the 14 LGS stage onwards had no effect on the 100-kernel weight.

Grain yield is a measure of the effectiveness of the application of *S. maydis* inoculum to the plants. Reduced yields before and after the 14 LGS and anthesis suggested a significant stalk rot influence on yield. This was supported by the prolificacy, lodging, grain moisture and days to physiological maturity results. This technique can therefore be used to screen germplasm for stalk rot resistance by inoculating the maize before LGS 10 and/or after 50% anthesis.

Overall, the optimum growth stage for inoculation was not consistent but the 12 - 14 LGS appeared to be the optimum period for inoculation to take place. Inoculation at 50% anthesis also resulted in a significant increase in diseased grain and affected a number of other agronomic

traits. Overall, the best inoculation technique was the use of 5 - 10 g fine inoculum applied at the 12 - 14 LGS.

The principle of inoculating maize in the whorl is not new but has primarily been used to introduce foliar pathogens onto the maize plants (Ayers *et al.*, 1970; Burnette and White, 1985; Bowen and Pedersen, 1988; Pataky, 1994). Most of these techniques were based on the use of conidial suspension but some researchers used infected plant material to induce leaf disease epidemics. Warren and Onken (1981) and Warren and von Qualen (1984) used conidial suspensions in the leaf whorl of maize plants to increase ear and stalk rot infection. The method developed in South Africa of inoculating plants with *S. maydis* inoculum, derived from colonised ears of the previous season, was a breakthrough for seed companies seeking to conduct large scale screening of maize germplasm. The advantages are:

i) the easy collection of inoculum
ii) it is cost effective
iii) allows for easy storage of inoculum
iv) is easy to apply and a simple process
v) large numbers of plants can be inoculated in a short space of time
vi) no laboratory facilities or highly trained staff are needed during the process of inoculum collection, processing and inoculation
vii) the stage of inoculation can be varied to induce ear rot and/or stalk rot.

Further, the inoculum is in a form that is not greatly influenced by the environment and will produce conidia as soon as the environment is suitable. Desiccation is not a problem, as it is with conidial suspensions, because the fungus can re-grow from the inoculum when conditions are suitable for sporulation and infection.

An improvement that would be desirable for accurate pathological studies is the production of pure *S. maydis* inoculum on sterilized maize or sorghum kernels in the laboratory to improve the consistency of application (concentration) and reduce the possibility of interaction of this pathogen with other organisms present in naturally infected ears. Flett and McLaren (1994) used pure *S. maydis* inoculum produced on maize kernels to inoculate maize hybrids to specifically determine their response to *S. maydis* ear rot. McLennan (1991) and Bensch (1995) have successfully used the technique developed in this study for ear rot inoculation.

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CHAPTER 3

Assessment of Maize Hybrids for Ear Rot Infection in South Africa[3]

ABSTRACT

The assessment of a maize hybrid's resistance to ear pathogens had largely been ignored in South Africa until significant ear rot epidemics occurred, starting in the 1985/86 growing season. These epidemics suddenly resulted in intensive research being initiated on hybrid response to ear pathogens. The object of this study was to compare a variety of methods for ear rot assessment, looking for one which was accurate, practical and efficient. The results showed that different methods of ear rot assessment could be used, depending upon the degree of accuracy and reliability of the information needed. There was significant variation in a person's ability to visually assess ear rot consistently and accurately (maize research experience was very important). Maize grain colour affected a person's ability to accurately visually assess the amount of diseased grain present.

3.1 INTRODUCTION

Maize ear rot epidemics in South Africa during the 1980's resulted in considerable research on maize ear rot being initiated in South Africa (Rheeder, 1988; Flett, 1990; McLennan, 1991; Viljoen[4], pers. comm.). Although a significant amount of research has been undertaken on maize ear rot in other regions of the world (Hooker, 1956; Koehler, 1959; Wiser *et al.*, 1960; Villena, 1969; Thompson *et al.*, 1971; Warren and Shepherd, 1976; Sivasankar *et al.*, 1976; Warren, 1978; King and Scott, 1981; Mesterhazy and Kovacs, 1986) it was necessary to investigate the detail of the epidemic in South Africa. One of the main differences between maize ear rot research in South Africa and the Northern Hemisphere countries, is that in the Northern Hemisphere the pathogens involved are usually *Fusarium* rather than *Stenocarpella* species.

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3 Partly published in the *Proc. 8th S. Afr. Maize Breeding Symp.* (1989), Potchefstroom 1988, J.G. du Plessis (Ed.), Tech. Comm. No. 222, Dept. Agric. Tech. Services, Pretoria, RSA.

4 J.H. Viljoen, formerly Senior Manager: Product Services, Maize Board, P.O. Box 669, Pretoria 0001, RSA.

Recent epidemics, which started in 1985/86, emphasised the need to collect and present ear rot data as accurately as possible. Since then, the South African maize industry has become extremely aware of differences in resistance levels of commercial hybrids in the market. However, a problem at the time was that little scientifically and statistically valid ear rot data was available to researchers. This led to the investigation of more efficient and effective ways of collecting data on hybrid ear rot resistance by Nowell (1989a and 1989b). The objective of this study was to determine the most practical, efficient and accurate method of ear rot data collection.

3.2 Material and Methods

3.2.1 Ear Rot Assessment

In 1986/87, a triple lattice trial of 36 hybrids replicated three times at Greytown, KwaZulu-Natal, was planted on 8 October 1986 by hand with two kernels per planting hill. The plots were hand thinned to 49 950 plants ha^{-1} at the 5 leaf growth stage. The plot size was two rows of 4.4 m long, 0.9 m apart and with 20 plants per row. The trial was inoculated with *Stenocarpella* inoculum in order to increase the severity of the ear rot infection. Ears naturally colonised by *Stenocarpella* spp. during the previous season (laboratory tests showed that at least 98% of the *Stenocarpella* infection was *S. maydis* (Berk.) Sutton and the balance was *S. macrospora* Earle) were milled into a coarse meal. Approximately 3.5 g of this meal was placed in the whorl of each plant about ten days before anthesis.

Table 3.1: Outline of the experience and the principal duties of each researcher

Researcher	Years Service	Function
1	>20	Breeder
2	>15	Breeder
3	>10	Breeder/Entomologist
4	6	Production Research
5	3	Breeder
6	1	Breeder
7	5	Plant Pathologist
8	5	Breeder
9	4	Botanist

Four different methods of collecting ear rot data were investigated.

3.2.1.1 *Rating method*

At PANNAR SEED (PTY) LTD, Greytown, in excess of 30 000 plots are assessed for ear rot each season and the most common method of assessment is to rate each plot visually on a non-linear 0 - 5 scale (with 0 = no visual disease, 1 = 3 - 6% visually rotten ears, 2 = 6 - 12.5% visually rotten ears, 3 = 12.5 - 25% visually rotten ears, 4 = 25 - 50% visually rotten ears and 5 = >50% visually rotten ears). Only rotten ears with >10% of the ear rotten were counted. This method was usually used in the initial testing phase of maize inbreds and hybrids.

3.2.1.2 *Percentage diseased ears method*

The percentage diseased ears were determined by counting the number of maize ears with greater than 10% of the ear rotted and determined as a percentage of the total number of ears. This method is more time consuming than the rating method and relies on a visual assessment of the degree of infection of each ear without shelling the grain or breaking the ears in half. This method was usually used to access the degree of infection in the advanced testing phase of maize inbreds and hybrids.

3.2.1.3 *Percentage diseased kernels method*

The whole plot was shelled, mixed well, a sub-sample of 250 g drawn and then the diseased kernels visually separated from the healthy kernels. The diseased kernels were then weighed and the percentage diseased kernels determined. This was the most accurate method as it gave the actual disease as seen by the grain inspectors on delivery to the storage silos, the millers and the farmers, and there was no hidden disease factor.

3.2.1.4 *Separate fungi method*

A slightly less accurate method of visual determination of the causal organism was to categorise the infected ears into the following categories:

Stenocarpella (*Stenocarpella maydis* and *S. macrospora*) - a white rot usually starting at the base of the ear

Fusarium (*Fusarium moniliforme* Sheldon and *F. subglutinans* (Wollenw. & Reinking) Nelson, Tousson & Marasas) - a pink/salmon rot of individual kernels spread randomly over the ear

Gibberella (*Fusarium graminearum* Schawbe.) - a red rot usually starting from the tip of the ear

Stalkborer (fungal infection due to stalkborer damage - usually *Fusarium* species) and

Others - any other fungal disease, e.g. *Penicillium* species.

Each ear was visually inspected by shelling two complete rows by hand while inspecting the kernels for signs of hidden fungal infection. These categories were further divided into groups based on the percentage of the ear that was infected as follows:

% Stenocarpella	% Fusarium	% Gibberella	% Stalkborer	% Others
100 75 50 25 10	100 75 50 25 10	100 75 50 25 10	50 25 10	50 20

It was important to determine the amount of stalkborer damage as this significantly influences the incidence and severity of *Fusarium* due to secondary infection of the insect damaged grain. Rots caused by the fungi other than those mentioned above are important as they are also responsible for discoloured grain and disintegration of the cobs. The proportion rotten grain, as a percentage, in each category was determined.

At harvest the ears were placed at the beginning of each plot and nine maize researchers (Table 3.1) visually rated the trial before any of the other ear rot determination methods were undertaken.

All data was compared to the percentage diseased kernels as this is the official method of grading maize in South Africa and is also the most practical, accurate method of visually assessing the amount of ear rot. Laboratory analysis of the diseased grain was not undertaken due to costs, time, labour requirements and logistical problems associated with such an exercise.

These data were analysed by correlating, using GENSTAT v. 5.31, all the different methods of ear rot assessment with each other. Tables 3.3 - 3.5 were divided into essentially three groups of correlations :

Group 1 - Researcher vs Researcher (top left section of Tables). This allows comparisons between researchers ratings to determine their consistency.

Group 2 - Researcher vs assessment methods (bottom left section of Tables). This is used to determine the accuracy of the researchers ratings and to identify which attribute is most closely correlated with the researchers ratings.

Group 3 - assessment methods vs assessment methods (bottom right section of Tables). This is used to identify to most important assessment method and the accuracy associated with the various methods.

This format will be used to discuss the results. The hybrids were all analysed together and then

the analysis was repeated for the yellow- and white-grained hybrids separately.

3.3 RESULTS

3.3.1 All hybrids

In Table 3.3, all correlations between the researchers was highly significant (all correlation coefficients ≥ 0.190 were significant at the 5% level of significant; 107 degrees of freedom). However, there was variation between individual's ratings, with Researcher 6 consistently showing the least correlation with other researchers. Researcher 8 showed variation in correlation with other breeders and had the lowest correlations of the balance of the researchers.

All researchers, except Researcher 6, had good correlations with the calculated rating (RT) for ear rot. However, when the means of the researchers are compared to the mean calculated rating (RT), all researchers consistently underestimated the amount of ear rot present, particularly Researchers 1, 5, 6 and 9.

Stenocarpella-infected ears correlated well with the researchers' ear rot ratings. The mean for *Stenocarpella*-infected ears was far higher than for all the other categories of ear rot. Researcher 6 showed no correlation with *Stenocarpella*-infected ears. Most researchers showed a negative correlation between ratings and *Fusarium*-infected ears and stalkborer damaged ears.

Table 3.3: Correlation matrix for various methods of ear rot assessment for all hybrids during 1986/87

Category	Method of Assessment																		
	1*	2	3	4	5	6	7	8	9	RT	Steno	Fus	Gibb	Other	Total	Borer	RE	DG
1*	1.000																		
2	0.703	1.000																	
3	0.738	0.756	1.000																
4	0.657	0.617	0.636	1.000															
5	0.620	0.667	0.656	0.627	1.000														
6	0.446	0.424	0.561	0.388	0.368	1.000													
7	0.706	0.740	0.774	0.668	0.600	0.400	1.000												
8	0.570	0.652	0.599	0.636	0.757	0.458	0.643	1.000											
9	0.891	0.679	0.789	0.747	0.684	0.372	0.724	0.622	1.000										
RT	0.705	0.760	0.558	0.527	0.611	0.281	0.562	0.591	0.573	1.000									
Steno	0.731	0.706	0.592	0.547	0.467	0.138	0.585	0.501	0.676	0.721	1.000								
Fus	-0.154	-0.046	-0.271	0.216	0.022	-0.092	-0.034	-0.056	-0.099	0.021	-0.094	1.000							
Gibb	0.340	0.146	0.070	0.168	0.204	0.227	0.134	0.274	0.316	0.377	0.178	0.007	1.000						
Other	0.320	0.265	0.007	0.275	-0.011	0.067	0.172	0.133	0.194	0.100	0.334	0.230	-0.018	1.000					
Total	0.591	0.437	0.204	0.444	0.297	0.222	0.353	0.406	0.512	0.549	0.577	0.219	0.770	0.546	1.000				
Borer	0.122	-0.056	0.024	-0.089	0.034	-0.087	-0.164	-0.155	0.030	-0.024	0.015	-0.096	0.078	-0.240	-0.053	1.000			
RE	0.440	0.428	0.346	0.473	0.242	0.365	0.176	0.409	0.379	0.462	0.425	0.116	0.270	0.395	0.514	0.041	1.000		
DG	0.779	0.791	0.706	0.655	0.724	0.409	0.694	0.695	0.722	0.902	0.633	-0.014	0.459	0.136	0.590	-0.091	0.493	1.00	
Mean	2.194	3.806	3.611	3.796	2.630	2.824	3.546	3.852	2.713	4.519	17.17	0.11	2.17	0.51	19.96	0.33	35.57	42.12	

*1-9 = the 9 Researchers that rated the trial.
Steno = Infection by *Stenocarpella*
Other = Infection by fungi other than those mentioned above
RT = Calculated rating as determined from the number of rotten ears
Fus = Infection by *F. moniliforme* and *F. subglutinans*
Total = Total of all ear rot (except stalkborer)
RE = % rotten ears
Gibb = Infection by *F. graminearum*
Borer = Damage to ears by *B. fusca*.
DG = % diseased grain

The researcher's ratings and the percentage diseased ears did not correlate well and there was considerable variation between researchers. There was no correlation between Researcher 7 ratings and the percentage diseased ears. However, good correlation occurred between researcher's ratings and the percentage diseased grain, except Researcher 6 (although significant).

There was a close correlation between *Stenocarpella*-infected ears and the calculated rating (RT). "Other" ear rots also correlated with *Stenocarpella*-infected ears. *Gibberella*-infected ears correlated with the calculated rating.

The percentage rotten ears primarily correlated with *Stenocarpella*-infected ears but also with *Gibberella*-infected ears. The percentage diseased grain correlated with *Stenocarpella*-infected ears, *Gibberella*-infected ears and the percentage rotten ears.

3.3.2 Yellow hybrids only

In Table 3.4, Researcher 6 is the only person that showed poor correlations with other researchers (all correlation coefficients ≥ 0.237 were significant at the 5% level of significance; 68 degrees of freedom). The most consistent of the researchers was Researcher 4.

All correlations between researchers' ratings and the calculated rating were significant, but all researchers consistently underestimated the amount of ear rot on the yellow-grained maize. The highest correlations was obtained by Researcher 6. The lowest correlations were obtained by Researchers 4 and 5.

The *Stenocarpella*-infected ears showed the highest correlation with the researcher's ratings. The lowest correlations were obtained by Researchers 3, 4, 5, 7 and 9. The correlations between "Other" ear rots and researchers' ratings were often important. Stalkborer damage showed a negative correlation with researchers' ratings.

Researchers 3, 5, 7 and 9 ratings did not correlate with the percentage rotten ears. All researchers' ratings had a good correlation with the percentage diseased grain. The highest correlation with the percentage diseased grain was obtained by Researcher 6.

Table 3.4: The correlation matrix for various methods of ear rot assessment for the yellow-grained hybrids only (1986/87)

Category	Method of Assessment																	
	1*	2	3	4	5	6	7	8	9	RT	Steno	Fus	Gibb	Other	Total	Borer	RE	DG
1*	1.000																	
2	0.654	1.000																
3	0.639	0.736	1.000															
4	0.728	0.689	0.708	1.000														
5	0.704	0.714	0.729	0.700	1.000													
6	0.735	0.740	0.746	0.720	0.529	1.000												
7	0.643	0.706	0.716	0.740	0.789	0.592	1.000											
8	0.668	0.708	0.613	0.722	0.758	0.495	0.783	1.000										
9	0.859	0.652	0.718	0.776	0.804	0.595	0.672	0.698	1.000									
RT	0.736	0.770	0.608	0.554	0.536	0.832	0.672	0.698	0.600	1.000								
Steno	0.634	0.669	0.596	0.563	0.501	0.602	0.561	0.660	0.567	0.665	1.000							
Fus	0.100	0.168	-0.022	0.225	0.218	0.193	0.025	0.121	0.189	0.189	-0.028	1.000						
Gibb	0.511	0.203	0.247	0.506	0.315	0.444	0.357	0.476	0.620	0.518	0.202	0.369	1.000					
Other	0.565	0.617	0.311	0.416	0.366	0.386	0.344	0.476	0.307	0.375	0.411	-0.226	-0.061	1.000				
Total	0.799	0.598	0.480	0.729	0.545	0.678	0.570	0.748	0.772	0.749	0.600	0.274	0.833	0.432	1.000			
Borer	-0.191	-0.349	-0.318	-0.143	-0.119	-0.260	-0.554	-0.211	-0.142	-0.423	-0.168	0.237	-0.112	-0.156	-0.173	1.000		
RE	0.416	0.530	0.381	0.406	0.286	0.621	0.187	0.487	0.272	0.592	0.381	0.495	0.398	0.429	0.621	0.096	1.000	
DG	0.836	0.749	0.770	0.710	0.742	0.841	0.780	0.749	0.785	0.886	0.525	0.177	0.622	0.407	0.799	-0.351	0.567	1.00
Mean	3.159	3.870	3.536	3.696	2.696	2.710	3.406	3.870	2.681	4.551	18.27	0.11	2.13	0.50	21.01	0.31	36.50	44.27

*1-9 = the 9 Researchers that rated the trial.
Steno = Infection by *Stenocarpella*
Other = Infection by fungi other than those mentioned above

RT = Calculated rating as determined from the number of rotten ears
Fus = Infection by *F. moniliforme* and *F. subglutinans*
Total = Total of all ear rot (except stalkborer)

RE = % rotten ears
Gibb = Infection by *F. graminearum*
Borer = Damage to ears by *B. fusca*.

DG = % diseased grain

Stenocarpella ear rot was the predominant ear rot disease in the yellow-grained hybrids. Although, the incidence of *Gibberella* and Other ear rot was low, these diseases correlated with the calculated rating, *Stenocarpella* ear rot and the Total ear rot. Stalkborer damage was negatively correlated with most assessment methods and diseases.

The percentage rotten ears was closely correlated with all assessment methods and diseases, except with damage due to Stalkborer. Only *Fusarium* ear rot and Stalkborer damage were not correlated with the percentage diseased grain.

3.3.3 White hybrids only

Ear rot ratings of researchers in Table 3.5 showed considerable variation (all correlation coefficients ≥ 0.317 were significant at the 5% level of significance; 38 degrees of freedom). The ear rot ratings of Researcher 6 only correlated with Researcher 8 ratings and showed a tendency to be negatively correlated with other researchers ratings. The ear rot ratings of Researchers 4 and 8 had poor and variable correlation with the other researchers.

All researchers tended to underestimate the amount of ear rot when compared to the calculated ratings (RT in the Tables). The ear rot ratings of Researcher 6 and Researcher 8 had no correlation and poor correlation with the calculated ear rot rating, respectively. Researcher 6 was the only person not to have a good correlation with *Stenocarpella*-infected ears and the ratings of Researcher 8 were not well correlated with *Stenocarpella*-infected ears. The ear rot ratings of Researcher 6 showed a negative correlation with *Stenocarpella*, *Fusarium*, Other ear rot and Total ear rot assessments.

The percentage rotten ears did not correlate well with researchers' ear rot ratings in the white-grained maize. Only Researchers 1 and 4 had a good correlation, and Researcher 8 a poor correlation, with the percentage rotten ears.

The only disease to correlate with the calculated rating was *Stenocarpella* ear rot. *Gibberella* ear rot was correlated with the total ear rot and the damage due to stalkborer.

Table 3.5: The correlation matrix for various methods of ear rot assessment for the white-grained hybrids only (1986/87)

Category	Method of Assessment																	
	1+	2	3	4	5	6	7	8	9	RT	Steno	Fus	Gibb	Other	Total	Borer	RE	DG
1+	1.000																	
2	0.848	1.000																
3	0.866	0.882	1.000															
4	0.545	0.518	0.509	1.000														
5	0.623	0.622	0.727	0.667	1.000													
6	-0.022	-0.144	0.222	-0.277	0.370	1.000												
7	0.848	1.000	0.882	0.518	0.622	-0.144	1.000											
8	0.460	0.484	0.701	0.518	0.830	0.546	0.484	1.000										
9	0.935	0.812	0.874	0.696	0.696	-0.048	0.812	0.596	1.000									
RT	0.855	0.848	0.730	0.701	0.623	-0.194	0.848	0.460	0.772	1.000								
Steno	0.883	0.799	0.652	0.584	0.406	-0.434	0.799	0.237	0.874	0.806	1.000							
Fus	-0.357	-0.204	-0.524	0.218	0.000	-0.424	-0.204	-0.204	-0.342	0.051	-0.107	1.000						
Gibb	0.165	-0.091	-0.133	-0.516	-0.237	0.064	-0.091	-0.530	-0.092	-0.099	0.076	-0.190	1.000					
Other	-0.006	-0.249	-0.463	0.028	-0.331	-0.558	-0.249	-0.454	-0.014	-0.006	0.326	0.451	0.312	1.000				
Total	0.338	0.093	-0.152	0.008	-0.125	-0.478	-0.093	-0.444	0.198	0.258	0.542	0.284	0.657	0.845	1.000			
Borer	0.529	0.480	0.482	-0.014	0.250	0.166	0.480	-0.039	0.254	0.477	0.239	-0.318	0.546	-0.373	0.137	1.000		
RE	0.524	0.152	0.298	0.657	0.218	-0.251	0.152	0.164	0.636	0.378	0.570	-0.198	-0.077	0.380	0.305	-0.073	1.000	
DG	0.869	0.934	0.837	0.738	0.665	-0.206	0.934	0.545	0.856	0.962	0.839	-0.063	-0.228	-0.120	0.126	0.392	0.377	1.00
Mean	3.256	3.692	3.744	3.974	2.513	3.026	3.795	3.821	2.769	4.462	15.24	0.09	2.25	0.54	18.12	0.36	33.94	38.32

+1-9 = the 9 Researchers that rated the trial.
Steno = Infection by *Stenocarpella*
Other = Infection by fungi other than those mentioned above

RT = Calculated rating as determined from the number of rotten ears
Fus = Infection by *F. moniliforme* and *F. subglutinans*
Total = Total of all ear rot (except stalkborer)

RE = % rotten ears
Gibb = Infection by *F. graminearum*
Borer = Damage to ears by *B. fusca*.

DG = % diseased grain

Only *Stenocarpella* ear rot correlated with the percentage rotten ears and the percentage diseased grain.

3.4 DISCUSSION

Researchers' ear rot ratings generally showed good correlation amongst themselves, the exception being Researcher 6. The ear rot ratings of Researcher 6 (with the least experience on maize) showed poor correlation with other researchers with the hybrids overall, acceptable correlation with the yellow-grained hybrids and no correlation with the white-grained hybrids. With the white-grained hybrids, ear rot ratings of Researchers 4 and 8 showed poor correlation at times to other researchers ear rot ratings.

Generally, researchers' ear rot ratings correlated well with the calculated ear rot rating (RT), although the severity of disease was consistently underestimated. Researchers 5, 6 and 9 consistently underestimated ear rot more than the other researchers. Correlation between researchers and the calculated ear rot rating was best with the white-grained hybrids.

There was good correlation between researchers' ratings, the calculated rating and *Stenocarpella* ear rot. This correlation was to be expected as this was the predominant type of ear rot, regardless of grain colour. With the hybrids overall and the white-grained hybrids, *Gibberella* ear rot was seldom correlated with researchers' ratings. However, with the yellow-grained hybrids there was a correlation between *Gibberella* ear rot and researchers' ratings. "Other" ear rot was correlated with the *Stenocarpella* ear rot, especially with the yellow-grained hybrids. It is possible that this ear rot was atypical *Stenocarpella* infection resulting from late infection of the ear.

For the hybrids overall, the total rotten ears were correlated with all methods of assessment and Researcher's ratings. This pattern was also true for the yellow-grained hybrids but was not true for the white-grained hybrids. There was good correlation between the percentage diseased grain (taken as the absolute method, with everything else being compared to these data) with all methods of assessment. These results suggest that a significant proportion of the diseased grain is in fact "Hidden" ear rot. "Hidden" ear rot can be defined as kernel colonisation (discolouration of the embryo) which is not visible to the eye without removing the grain from the cob. The method of determining the percentage diseased grain is relatively time consuming

but most importantly, it detects the amount of "Hidden" ear rot present (not considered with the other assessment methods).

Most maize ears colonised by *F. moniliforme* in KwaZulu-Natal is as a result of stalkborer damage. In the case of this experiment, this correlation was not expected as ears with stalkborer damage, with or without *Fusarium* colonisation, were classed as Stalkborer-damaged ears. The reason for a negative correlation between *Fusarium* ear rot and Stalkborer-damaged ears and "Other" ear rot with the white-grained hybrids is not clear. It may mean that larvae do not feed on ears already infected by ear rot fungi.

Generally, the accuracy of the ratings and various methods of ear rot assessment was good but it was disconcerting to find a significant difference in accuracy between researchers' ratings and the white-grained and yellow-grained hybrids. However, this discrepancy is believed to be largely due to the type of colonisation, as the yellow-grained hybrids are more prone to "Hidden" *Stenocarpella* infection. This accounts for the lower correlation between *Stenocarpella* ear rot and the percentage rotted ears for the yellow-grained hybrids than for the white-grained hybrids.

The method of determining the percentage rotten ears is the most practical, yet accurate, method of ear rot assessment and was highly correlated with most breeders and methods of ear rot assessment. The lack of consistent correlation between the visual ratings of researchers suggests that accurate assessment of the percentage rotten ears can only be undertaken by examining each ear in turn. Grain would have to be removed from the base and tip of each ear to determine the presence of absence of "Hidden" ear rot.

A factor which played an important part in the accuracy of the individual researcher's ratings, was that of experience (see Table 3.1). Generally, accuracy was correlated with years of experience in maize research and hence ear rot assessment. Researchers 1 to 3 had more than 10 years of experience in maize breeding and tended to have higher correlation coefficients overall, especially with the calculated rating. Researchers 5, 6 and 9 showed the greatest variation in their rating and had 4 years or less experience in maize research. Researcher 6 only had one year of maize research experience and consistently showed the lowest correlation coefficients. Whenever possible, maize researchers with less than 4 years experience should be prevented from assessing important experiments or assisted by a person with greater maize

research experience. There was no correlation with the researchers' research function; eg., maize breeder, pathologist or entomologist (see Table 3.1).

In general, the white-grained hybrids in South Africa are significantly more resistant to ear rot than are the yellow-grained hybrids (Rheeder, 1988; Gevers *et al*, 1992). This was supported by the lower mean *Stenocarpella* ear rot for the white-grained hybrids compared to that for the yellow-grained hybrids.

The different methods of assessment all served a specific purpose and were relevant under certain circumstances. When general accuracy is required and large numbers of assessments have to be undertaken is a short space of time, then a visual rating of ear rot is acceptable. This method of assessment can be used for preliminary trials. If a more accurate method of assessment is required, then the number of ears with greater than 10% of the ear rotted can be counted and then converted to a percentage of the total number of ears. This method of assessment can be used for relatively advanced trials where more accurate data is required. The most accurate method directly applicable to the maize industry is that of determining the percentage diseased grain by shelling the ears and then undertaking a visual assessment of the grain. As this method is labour intensive and time consuming, so it should only be used on trials where ear rot levels and importance of the trials warrant this type of intensive effort. The separate-fungi assessment method indicates the proportions of causal fungi and the primary pathogen resulting in diseased kernels but is extremely time consuming. This method would only be used in pathological studies and on a limited number of trials.

Koehler (1959) stated that it was necessary for one researcher to complete ear rot assessments in a whole trial, due to variation in assessment scores between researchers. However, a more desirable way is to have a different researcher assess each replication in order to reduce any error or discrepancies between researchers. This trial has shown that training researchers to undertake accurate ear rot assessment is important, especially to improve correlation between researchers and improve their accuracy and consistency. This training would involve identification of the causal fungi, the different methods of assessment and correct any natural bias. It would be necessary to refresh the researchers in ear rot assessment at the beginning of each harvest season to improve uniformity in assessment and should be undertaken once a month during harvest (usually over a four month period).

Further research is required to determine whether these trends are consistent over seasons and to establish the underlying causes of these patterns in ear rot assessment. There is a need to determine the influence of maize ear rot incidence, severity, causal pathogen, planting date, symptom expression/resistance and consistency of individuals on the accuracy and method of ear rot assessment.

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CHAPTER 4

Responses of Maize Hybrids to Ear Rot Infection in South Africa

ABSTRACT

Following the ear rot epidemics of 1986 onwards, accurate and reliable determination of hybrid response to maize ear pathogens was required. Initial research showed that a single assessment did not give an accurate assessment of the response of each hybrid, when grown under a wide variety of environmental conditions. The object of this research was to develop methods of presenting multi-locational ear rot information in an accurate and effective way that could be understood by farmers and researchers alike. This study showed that hybrid response to ear rot, primarily caused by *Stenocarpella maydis*, was not linear in nature under increased inoculum pressure and that many factors affected the expected response. Frequency tables were employed to indicate the frequency of an expected response for a given hybrid. This was an effective technique for the examination of hybrid response at various locations and over seasons. Non-linear regression and the Z-score methods of analysis were also tested on the same set of data. The overall result was that hybrid response to ear rot can now be accurately and reliably evaluated from a relatively small set of trials.

4.1 INTRODUCTION

Maize ear rot became a major problem in South Africa in recent years, resulting in a considerable amount of research being initiated in South Africa (Rheeder, 1988; McLennan, 1991; Flett, 1992; Flett and McLaren, 1994; Farwell[5], unpublished; Cronje[6], pers. comm.; Nowell, unpublished). Although a significant amount of research has been undertaken on maize ear rot in other regions of the world (Hooker, 1956; Koehler, 1959; Wiser *et al.*, 1960; Villena, 1969; Thompson *et al.*, 1971; Sivasankar *et al.*, 1976; Warren and Shepherd, 1976; Warren, 1978; King and Scott, 1981; Mesterhazy and Kovacs, 1986), it was also necessary to investigate the epidemic in South Africa as climatic conditions and maize germplasm are different. One of

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the main differences between research in South Africa and the countries in the Northern Hemisphere, is that the dominant ear rot pathogens in the Northern Hemisphere are *Fusarium* spp., whereas they are *Stenocarpella* spp. in South Africa.

Due to the relatively low incidence of ear rot in South Africa in the past, research on maize ear rot fungi was not of primary concern to the South African maize breeders and research personnel. The recent epidemics which started in 1986/87 emphasised the need to determine maize hybrid response to ear rot pathogens as accurately as possible. Subsequently, members of the South African maize industry have become extremely aware of differences in ear rot resistance levels of commercial hybrids in the market. A major problem initially was that there was very little scientifically and statistically valid ear rot data for South Africa was available. When such data was available, it was often in a form that was neither meaningful or of practical benefit to the maize industry because of the difficulty in interpreting them. This problem led to the development of more efficient and effective ways of collecting and presenting data on hybrid ear rot resistance by Nowell (1989a and 1989b) and subsequently Flett and McLaren (1994).

The objective of this study was to investigate accurate and practical methods of ear rot data presentation that can be easily understood by farmers.

4.2 Material and Methods

In 1986/87 and 1987/88, thirty trials from the Pannar Seed (Pty) Ltd national trials were used to study hybrid responses across seasons and geographical regions. All trials had the percentage colonised grain determined in at least one replication and had mean disease levels greater than 8.0% diseased grain. Trials with mean disease less than 7.0% were excluded due to the probability of late stalkborer infestations (caused by *Busseola fusca* Fuller and *Chilo partellus* Swinhoe) resulting in *Fusarium moniliforme* Sheldon being the main cause of ear rot, especially in the Western part of the maize producing area. In 1987/88, due to logistical limitations as a result of the relatively large number of trials infected by ear rotting fungi, only one replication of off-station trials were brought in for diseased kernel determinations. On the research stations at least two replications were used in both seasons.

In 1991 a trial for the purpose of screening hybrids for ear rot resistance was initiated by the Agricultural Research Council (ARC) and the Maize Industry at three localities in South Africa. This trial was an extension of the Phase II commercial hybrid series co-ordinated by the ARC throughout the maize producing region. Each site contained 49 entries replicated three times. The sites were at Greytown (KwaZulu-Natal), Petit/Delmas (Mpumalanga) and Potchefstroom (North West Province). Table 4.1 summarises the trial information at these locations.

Table 4.1: A summary of the ARC trials planted for ear rot screening between 1991 and 1994

Location	Company / Institute	Year	Plot Size
Greytown	PANNAR (Pty) Ltd	1991/92	4 X 4.4m X 0.9m
Petit	Cargill (Pty) Ltd		2 X 10m X 0.84m
Delmas	SENSAKO (Pty) Ltd		2 X 10m X 0.9m
Potchefstroom	ARC		1 X 20m X 2.15m
Greytown	PANNAR (Pty) Ltd	1992/93	4 X 4.4m X 0.9m
Petit	Cargill (Pty) Ltd		2 X 10m X 0.84m
Delmas	SENSAKO (Pty) Ltd		2 X 10m X 0.9m
Potchefstroom	ARC		1 X 20m X 2.15m
Greytown	PANNAR (Pty) Ltd	1993/94	4 X 4.4m X 0.9m
Petit	Cargill (Pty) Ltd		2 X 10m X 0.84m
Delmas	SENSAKO (Pty) Ltd		2 X 10m X 0.9m
Potchefstroom	ARC		1 X 20m X 2.15m

Each whole plot was subdivided into two sub-plots, one for inoculation with *Stenocarpella maydis* (Berk.) Sutton and the other as the non-treated control. Inoculum was produced by the ARC by inoculating sterilised maize whole kernels with pathovars of *S. maydis*, from the regions that trials were to be planted, and allowing the fungus to grow at 26°C for 9 weeks. The colonised kernels were then air dried and milled into a relatively fine meal. Approximately 3.5 g of inoculum was placed in the whorl of each plant to be infected, using a coffee dispenser (Nestlé [SA] [Pty] Ltd, Durban, South Africa), approximately 14 days before 50% anthesis. At harvest the percentage *S. maydis*-infected ears was determined by physically examining each ear and counting ears with greater than 10% rotten grain, and then determining the number of infected ears of the total number of ears as a percentage. The percentage diseased grain were determined by shelling the whole plot sample, drawing a 500 g sample, visually separating the diseased grain from the healthy grain and then calculating the percentage rotten grain based on

the mass of the two fractions.

Four methods were employed to find the most effective and practical method of presentation of hybrid response to ear rot. These were:

i) Data expressed as a percentage of the mean infection for the trial

ii) Data presented as frequency tables

iii) The standardised Z variate method

iv) Regression analysis of hybrid response to *Stenocarpella* ear rot infection.

The standardised Z variate (Z-score) is determined by dividing the ear rot value by the standard error for the trial (Steel and Torrie, 1981; Gomez and Gomez, 1984; Fowler and Cohen, 1990; Pataky and Eastburn, 1993). The Z-scores are then used to categorise hybrid response into resistance/susceptibility groups. This is subjective and the range in the Z-scores was slightly different each season. However, in general those hybrids with a Z-score > 1.1 were categorised as ear rot susceptible, and those with Z-scores < -1.1 were classed as ear rot resistant. The Bayesian least significant difference test was not available in Genstat 5.32 or Statsgraphics 4.0 as an additional tool as used by Pataky and Eastburn (1993) to further improve the classification of hybrids into resistance/susceptibility categories.

The Z-score was used to divide the hybrids into the following resistance categories:

R	Resistant to *S. maydis* ear rot
MR	Moderately resistant to *S. maydis* ear rot
M	Intermediate in *S. maydis* ear rot response
MS	Moderately susceptible to *S. maydis* ear rot
S	Susceptible to *S. maydis* ear rot.

As there are no maize hybrids in South Africa that are considered highly resistant to *S. maydis* ear rot, Z-scores were divided in such a way as to give few R responses.

4.3 RESULTS

The data from the PANNAR SEED trials are presented in Section 4.3.1 and 4.3.2 as a greater number of seasons and number of locations were available. Tables are based on the percentage infected grain as a measure of the amount of ear rot. The data from the ARC Phase II commercial hybrid trials was used in Section 4.3.3 and 4.3.4.

4.3.1 Hybrid response relative to the trial mean

Due to natural variations of hybrid response to ear rot over locations and seasons, it is desirable that the poorest and best performing hybrids can be identified easily. This method is a non-statistical method that was employed to allow trends to emerge from trial data over locations and years. By standardising the data, using the trial mean, greater differentiation between hybrids occurs and the extremes in hybrid response to ear rot are more marked.

Table 4.2: The incidence of diseased grain, expressed as a percentage of the mean percentage kernel colonisation for the trial, of seven selected hybrids at five localities during the 1986/87 season

Hybrid	Bergville	Utrecht	Greytown	Normandien	Carolina	Mean
CRN 4502	189.4	155.8	159.0	263.6	145.7	182.7
PAN 6549	76.8	57.8	77.1	64.8	48.0	64.9
PAN 473	65.4	73.8	75.4	45.5	139.4	79.9
PAN 6514	42.2	38.8	45.9	30.7	78.9	47.3
PAN 6528	88.2	155.8	86.1	102.3	166.3	119.7
RS 5206	58.9	240.8	69.7	121.6	72.6	76.3
SNK 2244	162.6	240.8	92.6	280.7	162.3	187.8
Rel. Mean[1]	100.0	100.0	100.0	100.0	100.0	100.0
Mean	24.6	14.7	12.2	8.8	17.5	15.6

[1] The mean percentage infected kernels for the trial is taken as being 100% and all data is determined as a percentage of the mean.

Table 4.3: The response of seven maize hybrids to ear rot infection at a single locality for four seasons, expressed as a percentage of the trial mean for 1985/86 to 1989/90

Hybrid	1985/86	1986/87	1987/88	1988/89	1989/90
CRN 4502	144	159	106	122	-[2]
PAN 6549	81	77	83	58	125
PAN 473	125	75	127	101	76
PAN 6514	81	45	146	60	-
PAN 6528	62	86	130	145	134
RS 5206	62	70	98	58	47
SNK 2244	81	93	101	-	-
Rel. Mean[1]	100	100	100	100	100
Mean	11.7	12.2	18.2	9.2	8.4

[1] The mean percentage infected kernels for the trial is taken as being 100% and all data is determined as a percentage of the mean.

[2] The hybrid was not in the trial during the given growing season.

Table 4.2 shows the response of seven maize hybrids to ear rot colonisation over seven different environments. Although the response of some hybrids is consistent over locations, hybrids such as PAN 6528 and RS 5205 exhibited a large variation in ear rot response. The largest variation in hybrid response to ear rot between hybrids, occurred at the site with the lowest mean disease level (Normandien). From Table 4.2, it is possible to identify those hybrids that will always have less ear rot than most other hybrids, and those hybrids likely to have severe ear rot problems should the environment be conducive to infection. A number of the hybrids do not fit into either category.

Table 4.3 shows that hybrid response to ear rot colonisation at one location (Greytown) is not consistent over seasons. Only RS 5206 had consistently less ear rot and CRN 4502 had consistently more ear rot than the mean of the trial over 5 seasons. In Table 4.2, SNK 2244 appeared to be one of the most susceptible hybrids to ear rot, whereas Table 4.3 shows this hybrid to have better-than-average ear rot resistance. Most hybrids performed poorly or well in at least one seasons out of five.

4.3.2 Frequency table analysis

Due to the variation in response of hybrids to ear rot infection reflected in Tables 4.2 and 4.3, it was decided to determine the frequency of hybrid response to ear rot infection. By using this method, it is possible to compare hybrids against each other, in geographical regions and over seasons. Table 4.4 gives the ear rot response of PAN 6549 in different geographical regions. This can be compared to the same information for PAN 6528 in Table 4.5 and for CRN 4502 in Table 4.6. Table 4.4 shows that overall PAN 6549 gets less ear rot than the trial mean but there is considerable variation in response between regions.

Table 4.4: The proportion of trials (%) in ear rot disease classes from different regions for PAN 6549 during 1986/87 and 1987/88

	1986/87 & 1987/88							
Region	Relative percentage disease							No. trials
	<50	50-70	70-90	90-110	110-130	130-150	>150	
Overall	10	23	33	10	17	3	3	30
KwaZulu-Natal	10	33	38	5	10	5		21
Mpumalanga	25				75			4
North West			40	40			20	5

Table 4.5: The proportion of trials (%) in ear rot disease classes from different regions for PAN 6528 during 1986/87 and 1987/88

	1986/87 & 1987/88							
Region	Relative percentage disease							No. trials
	<50	50-70	70-90	90-110	110-130	130-150	>150	
Overall	10	7	23	20	10	17	17	30
KwaZulu-Natal	3	3	24	24	3	24	14	21
Mpumalanga			25		25		50	4
North West	40	20	20	20				5

Overall, PAN 6528 displayed an average to an above average amount of ear rot, but appears to get considerably less ear rot in the North West Province than in the Mpumalanga and KwaZulu-Natal Provinces (Table 4.5). From Table 4.6 it can be seen that CRN 4502 is highly susceptible to ear rot and had more ear rot than the trials mean in at least 40% of the plantings regardless

of which region it was planted. Hybrids such as CRN 4502 should be avoided in regions that have a high probability of ear rot.

Table 4.6: The proportion of trials (%) in ear rot disease classes from different regions for CRN 4502 during 1986/87 and 1987/88

1986/87 & 1987/88 Combined								
Region	Relative percentage disease							No. trials
	<50	50-70	70-90	90-110	110-130	130-150	>150	
Overall	3	13	17	10	10	13	30	30
KwaZulu-Natal	5	14	10	14	10	14	33	21
Mpumalanga		25	25			50		4
North West		20	40		20		20	5

Tables 4.7 and 4.8 show the difference in response to ear rot infection by CRN 4502 in consecutive seasons. In 1986/87 (when the ear rot incidence was highest), the level of ear rot in CRN 4502 was always much higher than the mean of all hybrids. This was independent of which province the hybrid was planted (Table 4.7). In 1987/88 the ear rot inoculum pressure was lower and CRN 4502 did not always do worse than the mean, although this was often the case. During this season there appeared to be a tendency for the hybrid to have less ear rot than other hybrids in the Mpumalanga and North West Provinces (Table 4.8).

Table 4.7: The proportion of trials (%) in ear rot disease classes from different regions for CRN 4502 during 1986/87

1986/87								
Region	Relative percentage disease							No. trials
	<50	50-70	70-90	90-110	110-130	130-150	>150	
Overall						14	86	7
KwaZulu-Natal						17	83	6
Mpumalanga						100		1
North West								0

Table 4.8: The proportion of trials (%) in different ear rot disease classes from different regions for CRN 4502 during 1987/88

1987/88								
Region	Relative percentage disease							No. trials
	<50	50-70	70-90	90-110	110-130	130-150	>150	
Overall	4	17	22	13	13	13	13	23
KwaZulu-Natal	7	20	13	20	13	13	13	15
Mpumalanga		33	33			33		3
North West		20	40		20		20	5

4.3.3 Z-score analysis

Z-scores were determined for the mean percentage rotted ears and the mean percentage diseased grain for 1991/92, 1992/93 and 1993/94. Table 4.9 shows selected hybrids and the ear rot resistance categories allocated to the Z-score. The resistance categories of Pataky and Eastburn (1993) are similar to those allocated in South Africa, except that they allowed for greater differentiation between hybrids. The category of "ear rot resistant (R)" was not allowed for in South Africa, as even the least ear rot susceptible hybrid could still exhibit significant levels of ear rot infection. However, of the hybrids shown, PAN 6479, PAN 6480, RS 5206, NS 9100 and SNK 2665 rated consistently better than the other hybrids (regardless whether the categorisation was based on percentage colonised ears or percentage diseased grain), and consistently fell into the moderately resistant class. According to the classification of Z-scores used by Pataky and Eastburn (1993), most of these hybrids should be classed as ear rot resistant. CRN 4502 was consistently classed as susceptible.

When the data from the percentage diseased ears is examined (Table 4.9), HL 8 (present in two out of three seasons) was found to be moderately resistant in 1991/92 but moderately susceptible to ear rot in 1992/93. PAN 6364 was more resistant to ear rot in 1991/92 than in either 1992/93 or 1993/94. SNK 2950 was more susceptible to ear rot in 1991/92 than in either 1992/93 or 1993/94. PAN 473 showed considerable variation in the Z-score although the hybrid still maintained the same resistance category. PAN 6528 appeared to become more resistant (a smaller Z-score) each season and moved up a resistance category.

Table 4.9: Categorisation into ear rot resistance groups of selected hybrids for the percentage diseased ears and the percentage diseased grain during the 1991/92, 1992/93 and 1993/94 seasons based on Z-scores

Hybrid	1991/92			1992/93			1993/94		
	Z-score	A	B	Z-score	A	B	Z-score	A	B
% Diseased Ears									
CRN 3414	0.576	M	MS	0.858	MS	MS	-0.134	M	M
CRN 4502	2.409	S	S	1.817	S	S	1.767	S	S
HL 8	-1.569	MR	R	0.798	MS	MS	----	----	----
NS 9100	-1.500	MR	R	-1.062	MR	R	-0.806	MR	R/MR
PAN 473	0.519	M	MS	-0.401	M	MR	-0.231	M	M
PAN 6364	0.169	M	M	0.875	MS	S	1.361	MS	S
PAN 6479	-0.826	MR	MR	-0.814	MR	R/MR	-0.429	M	MR
PAN 6480	-1.662	MR	R	-0.491	M	MR	-0.820	MR	R
PAN 6528	1.381	MS	S	0.948	MS	S	0.272	M	M
PAN 6578	-0.756	MR/M	MR	-0.132	M	M	-0.360	MR	MR
RS 5206	-1.236	MR	R	-0.933	MR	R	-0.914	MR	R
SNK 2665	-0.967	MR	MR	-0.920	MR	R	-0.747	MR	MR
SNK 2950	1.191	MS	S	0.057	M	M	0.133	M	M
% Diseased Grain									
CRN 3414	0.005	M	M	0.711	MS	MS	0.153	M	M
CRN 4502	1.503	S	S	1.111	S	S	1.286	S	S
HL 8	-1.147	MR	R	0.503	MS	MS	----	----	----
NS 9100	-1.133	MR	R	-1.012	MR	R	-0.549	MR	MR
PAN 473	-0.125	M	M	-0.668	M	MR	-0.274	M	M
PAN 6364	0.056	M	M	0.738	MS	MS	0.946	MS	S
PAN 6479	-0.766	MR	MR	-0.742	MR	MR	-0.139	M	M
PAN 6480	-1.103	MR	R	-0.320	M	MR	-0.735	MR	MR
PAN 6528	1.053	MS	MS/S	0.840	MS	S	0.471	M	MS
PAN 6578	-0.094	M	M	0.312	MS	MS/M	-0.550	MR	MR
RS 5206	-0.847	MR	MR	-0.483	M	MR	-0.839	MR	R
SNK 2665	-1.076	MR	R	-1.041	MR	R/MR	-0.850	MR	R
SNK 2950	0.931	MS	MS	0.295	MS	MS/M	-0.068	M	M

A = resistance category as determined by hybrid response in South Africa
B = resistance category based on the method used by Pataky and Eastburn (1993).

Z-scores and resistance classes based on the percentage diseased grain were similar to those based on the percentage diseased ears. However, SNK 2950 responded differently as the hybrid

was more resistant to ear rot infection in 1993/94 than in either 1991/92 or 1992/93. The variation in Z-score for PAN 473 was considerably smaller when based on the percentage diseased grain. In general, there was less variation in Z-scores when based on the percentage diseased grain rather than the percentage diseased ears.

These data are based on the overall means of the hybrids for all locations. Further analysis based on individual seasons, locations (Table 4.10) and various combinations, resulted in a high variation in Z-scores and resistance/susceptibility categories. This variation can be clearly seen in the incidence of ear rot in CRN 3414, CRN 4502, PAN 6479, SNK 2665 and RS 5206. For this reason it was decided not to use this technique to analyse the data further.

Table 4.10: Categorisation into ear rot resistance groups of selected hybrids for the percentage colonised ears during the 1993/94 season based on Z-scores

	Petit		Delmas		Greytown		Potchefstroom	
Hybrid	**Z-score**	**Cat.**	**Z-score**	**Cat.**	**Z-score**	**Cat.**	**Z-score**	**Cat.**
CRN 3414	***-0.584***	***MR***	***0.640***	***MS***	***-0.324***	***MR***	***0.057***	***M***
CRN 4502	***-0.669***	***MR***	***2.227***	***S***	***1.036***	***S***	***4.580***	***S***
NS 9100	-0.617	MR	-1.229	MR	-0.159	MR	-1.776	R
PAN 473	-0.407	MR	-0.147	MR	-0.053	MR	-0.542	MR
PAN 6364	0.568	MS	1.328	S	0.611	MS	3.357	S
PAN 6479	***0.289***	***MS***	***-0.954***	***MR***	***-0.182***	***MR***	***-0.983***	***MR***
PAN 6480	-0.649	MR	-1.339	MR	-0.264	MR	-1.277	MR
PAN 6528	-0.152	M	-0.342	M	0.043	MR	2.166	S
PAN 6578	0.028	M	-0.514	M	-0.106	MR	-1.154	MR
RS 5206	***0.282***	***MS***	***-1.894***	***R***	***-0.497***	***MR***	***-1.398***	***MR***
SNK 2665	***0.377***	***MS***	***-0.653***	***MR***	***-0.607***	***R***	***-1.988***	***R***
SNK 2950	0.430	MS	-0.356	M	-0.015	MR	0.780	MS

Cat = resistance category as determined by hybrid response in South Africa.
Those hybrids in bold italics showed large variation in ear rot resistance response.

4.3.4 Regression analysis

Flett and McLaren (1994) found maize hybrid response to ear rot to be non-linear in nature and described these responses statistically. The mean percentage infection for the trial at a specific

site was taken as the ear rot or inoculum potential. This was then plotted against the mean infection for a specific hybrid at a particular location. Non-linear regression was then applied to each hybrid using the formula $Y=AX^b$, where Y = mean ear rot incidence within each hybrid and X = ear rot potential. By applying non-linear regression analysis, many of the anomalies of hybrids not fitting expected responses could be accurately explained.

Hybrid response could be divided into three categories:

i) Hybrids which showed a linear response to ear rot with increased inoculum pressure.

ii) Highly ear rot susceptible hybrids despite a low inoculum potential.

iii) Hybrids of varying degrees of ear rot resistance despite an increase in inoculum potential (Flett and McLaren, 1994).

Table 4.11: Relative response of hybrids (1992/93 and 1993/94 combined) to *Stenocarpella* ear rot at different inoculum potentials compared to the ear rot resistance groupings based on the Z-score for diseased ears for 1992/93 and 1993/94 (Anonymous, 1995)

Hybrid	Inoculum potentials						Z-score class [1]	
	5	10	15	20	25	30	92/93	93/94
CRN 3414	5.1	10.7	16.6	22.6	28.9	35.0	MS	M
CRN 4502	13.5	22.3	30.0	37.0	43.6	49.8	S	S
NS 9100	2.3	5.3	8.7	12.4	16.2	20.3	MR	MR
PAN 473	5.0	9.5	13.9	18.2	22.4	26.5	M	M
PAN 6364	10.2	17.7	24.4	30.6	36.2	42.2	MS	MS
PAN 6479	2.2	5.4	9.0	13.0	17.3	21.8	MR	M
PAN 6480	3.2	6.8	10.7	14.7	18.9	23.1	M	MR
PAN 6528	7.0	13.5	19.8	25.9	31.9	37.9	MS	M
PAN 6578	4.5	9.1	13.8	18.6	23.4	28.2	M	MR
RS 5206	2.8	6.0	9.4	13.0	16.6	20.2	MR	MR
SNK 2665	3.8	7.4	10.9	14.4	17.8	21.2	MR	MR
SNK 2950	4.6	9.5	1.7	19.9	25.2	30.5	M	M
PAN 6034[2]	5.0	10.3	15.8	21.4	27.0	32.7		
PAN 6140[2]	6.1	14.6	24.2	34.7	45.8	57.8		
PAN 6141[2]	1.6	4.3	7.7	11.6	16.0	20.7		
PHB 3427[2]	2.3	7.0	13.3	21.1	30.1	40.2		

[1] Z-score classes are from Table 4.9 and are used for comparative purposes.

[2] data based on 1993/94 season only and no Z-scores were determined.

Flett and McLaren (1994) showed that by fitting confidence limits to the ear rot disease curves, the optimum disease potential for screening hybrids to ensure maximum differentiation between genotypes was 17 - 20%. The differentiation between genotypes at very low or very high disease potentials was not sufficient to result in detectable differences.

This technique has been further developed to allow prediction of hybrid ear rot response at specific disease potential levels (Anonymous, 1995). This allows for the easy identification of hybrids that are susceptible at all inoculum potentials. In addition, should maize be planted in an area of high ear rot risk, the hybrids that are highly susceptible under high disease pressure can also be identified. In Table 4.11, CRN 4502 is shown to be highly susceptible to ear rot at all disease potentials. CRN 3414 exhibits more ear rot with increases ear rot inoculum. PAN 6480 has below average ear rot colonisation regardless of the inoculum potential. PHB 3427 and PAN 6140 were moderately ear rot resistant at the 5% inoculum potential but were more susceptible as inoculum levels increased, particularly PAN 6140 (one season's data only). However, other hybrids such as PAN 6141 and PAN 6034 exhibited normal response patterns.

4.4 Discussion

The most important fact to emerge from this study was that hybrid response to *S. maydis* ear rot was not consistent over locations and/or seasons, and even the most ear rot resistant hybrids showed severe *S. maydis* ear rot at times. The variation found was too large to be explained as experimental error, and hybrids with similar pedigrees usually responded in a similar way to ear rot. This meant that new strategies for determining hybrid response to ear rot and data presentation had to be developed. These strategies had to be easy to practical, accurate, simple enough to be used in large scale screening and understandable to the various role players within the maize industry. For this reason, the most simple techniques were started with and then developed further, or substituted, as necessary.

Expressing data as a percentage of the mean of the trial allows for easy assessment of a hybrids response to ear rot in previous seasons compared to the other hybrids. An advantage is that data are easily comparable over seasons as trends are being compared rather than actual data. However, the problem associated with this is that the inoculum pressure is unknown and therefore the specific performance of a hybrid cannot be predicted in a given area. This type of information will help researchers, marketing personnel and farmers alike, identify the

extremes of good and bad performers. This reduces the risk of a farmer planting an inherently ear rot susceptible hybrid and/ or can be used to identify hybrids that are more suited to areas where ear rot is endemic. If the relative mean of the hybrid over locations is used as a measure of ear rot resistance, then the variation exhibited by some hybrids is missed completely. It is for this reason that the response over as wide a range of locations as possible should be examined and not data means.

An improvement on this method of data collection and presentation is to present the information as a frequency of ear rot response category. This highlights the risk of planting a particular hybrid in a high risk ear rot area. This allows for more detailed analysis and is easier to interpret. However, hybrids with an unstable response pattern are still difficult to accurately classify for a specific location. An additional problem is that of not having an equal number of trials in each category that the trials are divided. This can be seen in Tables 4.4 - 4.8, where KwaZulu-Natal had 21 trials, North West 5 trials and Mpumalanga 4 trials. Although it is possible to get a response pattern with a low number of trials, the accuracy of the data can be questioned. This type of analysis does not have a statistical method of testing for significant differences. Such a method could be developed but would be complicated and time consuming. The limitations of this technique should be recognised, but it does provide a reasonably accurate and practical method of ear rot assessment and interpretation of the data to people who do not have access to methods of statistical analysis. It is easy to identify consistent ear rot susceptible and more ear rot resistant hybrids but is difficult to interpret hybrid ear rot responses that vary considerably. For this reason a more accurate statistical method of analysis is desirable.

Z-scores are effective in classifying disease hybrids into response categories but in the case of ear rot, variation was high for some hybrids. This means that although the overall classification can be made, some hybrids cannot be accurately classified. In addition, the technique showed that detailed analysis for each site and season resulted in increased variation in the disease response pattern of hybrids. For those hybrids that responded linearly with increased inoculum pressure, the Z-score analysis worked well.

The ear rot response patterns (based on Z-scores) were similar for data collected as either percentage diseased ears or percentage diseased grain. Although the variation in Z-scores between seasons was higher when based on the percentage diseased ears, the increased work required to determine the percentage diseased grain does not justify the small improvement in

consistency of these data.

Table 4.10 showed the high degree of variation found in the Z-scores and resistance categories when detailed ear rot responses were examined. This method is more suited to generally classifying resistance categories based on the mean of data. Another method of ear rot data analysis is needed to explain this variation in data.

In the above studies it became apparent that there were certain ear rot inoculum threshold levels above which the resistance of most hybrids rapidly becomes less effective. This indicated that hybrid response to ear rot colonisation was not linear. Flett and McLaren (1994) explained these non-linear responses using non-linear regression analysis. This is the only technique to account for ear rot response accurately for all hybrids over seasons and locations. Many of the discrepancies in other methods of ear rot data presentation are accounted for by this method. Flett and McLaren have developed their model and they can now predict a hybrid's response to ear rot disease at various inoculum pressures, which can be seen in Anonymous (1995). This allows advisors and farmers to more accurately select hybrids for an anticipated ear rot situation. A possible shortcoming of this system at present is the overall response of a hybrid to ear rot is determined and a regional or geographic effect is ignored or assumed to be non-significant. Tables 4.4 - 4.8 suggest that there is variation in hybrid response to ear rot between regions. This was not investigated further in this study as this is being done at present by Flett (Flett[7], pers. comm.).

A problem with this technique is that considerable statistical knowledge is required to analyse these data. For this reason the technique cannot be easily transferred to other data sets and uses, especially if statisticians and sophisticated software are not available. Some farmers have also expressed difficulty in following the concept and interpreting the information. Should the programme be made user friendly, significant use can be made of this technique. Education of the farmers is required to ensure these data generated are understood and therefore used correctly and effectively.

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CHAPTER 5

Ear Rot Incidence in Maize Hybrids at Various Plant Densities In KwaZulu-Natal, South Africa

ABSTRACT

No prior published evidence existed for a relationship between maize plant density and ear rot incidence. As there is a very large range in plant densities employed in South Africa (from 9 000 to over 75 000 plants ha^{-1}), trials were conducted to measure ear rot incidence at plant densities that were being routinely deployed on farms. In the first trial in 1986/87, some hybrids showed a very significant increase in ear rot at 36 000 plants ha^{-1}, from 18 000 plants ha^{-1} and then a significant decrease in ear rot incidence at 40 000 - 50 000 plants ha^{-1}, before again showing an increase in ear rot incidence with a further increase in plant density. The relationship between ear rot severity and plant density was not linear, except possibly above 60 000 plants ha^{-1}. The second trial was conducted in 1987/88 during an abnormally dry season and expected patterns in ear rot severity did not materialise. However, the initial trends were repeated in the trial in the 1989/90 season, although less clearly than in 1987/88. Ear rot incidence did not correlate with tiller production per plant, prolificacy, lodging or grain moisture.

5.1 INTRODUCTION

During the 1985/86 growing season in KwaZulu-Natal, South Africa, there was an unexpected rise in the incidence of ear rots in maize (*Zea mays* L.). This was the first indication in South Africa that there was an increasing ear rot problem, primarily caused by *Stenocarpella maydis* (Berk.) Sutton. It was not until the 1986/87 growing season that the maize-producing area as a whole suffered severe losses in yield and quality as a result of an ear rot epidemic (Nowell, 1992). This maize ear rot epidemic resulted in a considerable amount of research on maize ear rot being initiated in South Africa (McLennan, 1991; Flett, 1992; Flett and McLaren, 1994; Rheeder, 1988; Farwell[8], unpublished; Nowell, unpublished).

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There was no information available in the literature on the response of maize hybrids to ear rot under different plant densities, other than Koehler (1959) mentioning that ear rot incidence and severity increases with increased plant density. Plant densities in South Africa vary considerably and are determined by the mean average or distribution of the annual rainfall or the availability of water for irrigation. In the lower rainfall areas of the maize production region the plant densities are usually between 9 000 and 25 000 plants ha^{-1}. In the maize producing regions of higher rainfall, the plant populations vary from 36 000 - 50 000 plants ha^{-1}. Plant density under irrigation can be up to 90 000 plants ha^{-1}.

The only published information on the relationship between a maize disease and plant density, involved the pathogen *Cercospora zeae-maydis* Tehon and Daniels, which causes Grey Leaf Spot (GLS). Initial research on GLS on maize indicated that high plant densities were conducive in creating high humidity microclimates favourable for disease (Payne and Waldron, 1983; Ayers *et al.*, 1985). However, recent work by Smith (1989) and de Nazareno *et al.* (1993) found that less disease per plant occurred under high plant densities because of a "shielding" effect from spore interception in the more dense canopies than in plots in which canopies were more open. Rivera-Canales (1993) found that GLS was more severe in seed crops in which there was significant removal of leaf tissue during detasselling, and the removal of male rows opened up the canopy. These results showed that plant density does effect the incidence of a disease and a plant density effect on the incidence of ear rot was possible.

The objective of this study was to determine whether or not plant density influenced the incidence of maize ear rot under natural conditions.

5.2 Materials and Methods

Maize plant density trials were conducted in 1986/87, 1987/88 and 1988/89 at Greytown, KwaZulu-Natal, to assess maize hybrid grain yield and ear rot response. No inoculum was applied because ear rot naturally and consistently occurred at this site The experimental plots had been under maize monoculture for at least six years and were prepared by mouldboard ploughing and discing the seedbed. Fertilizers were applied at a rate sufficient for a 10 t ha^{-1} grain crop based on the recommendations of the Cedara Fertilizer Advisory Service. Urea was broadcast before planting and disced into the soil and the other fertilizer was band placed before hand planting took place. During soil preparation EPTC (720 g ℓ^{-1}, Zeneca SA (Pty) Ltd) was

incorporated at a rate of four ℓ ha^{-1} to control grass weeds. Shortly after planting, a combination of atrazine and terbuthylazine (both at 250 g active ingredient, Kombat Chemicals [Pty] Ltd) at a rate of six ℓ ha^{-1} was applied to control broadleafed weeds. Stalkborer granules (25 g kg^{-1} carbaryl, Kombat Chemicals (Pty) Ltd) were applied by hand at the first signs of African stalkborer leaf damage caused by *Busseola fusca* Fuller.

1986/87 Experiment

This trial consisted of nine commercial maize hybrids planted as a factorial design with plant densities of 18 000, 36 000, 54 000 and 72 000 plants ha^{-1}. The plot size was two rows 6 m long, 0.9 m apart and the experiment was replicated three times. Two kernels per planting hill were planted. Plants were thinned by hand to the required plant density at the seven-leaf growth stage. The trial was planted on 10 November 1986 and 50% silk emergence was on 07 February 1987. The trial was harvested on 25 June 1987.

Although December, 1986 was a relatively dry month, the months of January, February and April, 1987 had a significantly higher rainfall than the 10-year average for these months. March, 1987 was a relatively dry month that resulted in some drought stress of the maize.

1987/88 Experiment

This trial consisted of twelve commercial maize hybrids planted as a factorial design with eight plant densities of 18 000, 26 000, 34 000, 42 000, 50 000, 58 000, 66 000 and 74 000 plants ha^{-1}. The plot size was two rows of 6 m long, 0.9 m apart and the experiment was replicated three times. Two kernels per planting hill were planted. Plants were thinned by hand to the required plant density at the seven-leaf growth stage. The trial was planted on 16 November 1987 and 50% silk emergence was on 08 February 1988. The trial was harvested on 20 May 1988.

The rainfall during January, 1988 was relatively low, resulting in moisture stress in the maize. The months of February and March were near average relative to the 10-year rainfall average.

1988/89 Experiments

A trial was monitored in which four hybrids (four single-cross female parents of four-way cross hybrids) were compared at five different plant densities of 34 000, 42 000, 50 000, 58 000 and 66 000 plants ha^{-1}. The trial was planted as a factorial design on 8 October, 1989, and the plot size was two rows of 6 m long, 0.9 m apart and the experiment was replicated twice. Two kernels per planting hill were planted. Plants were thinned by hand to the required plant density at the seven-leaf growth stage. The maize reached 50% silk emergence on 2 January, 1989 and was harvested on 14 May, 1989.

During 1989, the months of January and March had rainfal which was ideal for ear rot infection and development. However, February and April were relatively dry months, resulting in some moisture stress of the maize.

Although the primary objective was grain yield assessment, ear rot assessment was undertaken by shelling the whole plot, and visually separating out diseased grain, from a 500 g sample in 1986/87 and from a 250 g sample (sample size was reduced for logistical purposes) in 1987/88 and 1988/89. The percentage diseased grain was determined based on the mass of diseased kernels in the whole sample. In all seasons, laboratory analysis showed that the primary pathogens were *Stenocarpella* species, mainly *S. maydis* (Berk.) Sutton, which was responsible for more than 98% of the ear rot. The method of visually assessing the amount of diseased grain is used to grade maize on delivery from farmers to maize storage silos, and determines the price per tonne for the farmers.

These data were analysed using GENSTAT 5.31 (Lawes Agricultural Trust, Rothamsted Experimental Station, United Kingdom). No transformation of data was undertaken. Tests of significance were determined using Fischer's L.S.D. test of significance.

5.3 Results

White-grained hybrids are indicated by hybrid codes that end in an uneven digit, e.g. PAN 6549. Yellow-grained hybrids are indicated by hybrid codes that end in an even digit, e.g. PAN 6552. For illustrative purposes, four representative hybrids were selected from these data in each Table and graphed to show trends. During the 1986/87 and 1987/88 seasons, the four hybrids

(PAN 6330, PAN 6528, PAN 6549 and PAN 6552) were common to the trials. Unfortunately, a set of four hybrids (PNF 6429, PAN 6459, PNF 6549 and PNF 6552) which were different from these tested in 1988/89 had to be used.

1986/87 Experiments

Results for the 1986/87 season are presented in Table 5.1 - 5.3 and Figures 5.1 - 5.2.

Table 5.1: Percentage *Stenocarpella*-diseased grain for the maize hybrids at plant densities during 1986/87

	Plant density				
Hybrid	**18 000**	**36 000**	**54 000**	**72 000**	**Mean**
PAN 6549	9.6	10.6	11.0	17.1	12.1 a
PAN 6429	18.3	10.5	15.4	13.4	14.4 a
PAN 6557	13.3	21.9	25.3	25.7	21.6 b
PAN 6334	27.9	30.7	22.3	30.4	27.8 c
PAN 6330	16.5	29.8	30.9	35.3	28.1 cd
PAN 6528	20.8	39.8	26.1	30.7	29.4 cd
PAN 6434	29.9	24.2	26.4	41.8	30.6 cd
PAN 6552	29.5	36.0	29.9	30.8	31.6 d
PAN 394	40.3	40.3	24.6	45.7	37.7 e
Mean	22.9 a	27.1 b	23.6 a	30.1 b	25.9

A summary of the ANOVA results.			
Main Effect			
	Hybrids	F = 66.38	P = <0.001
	Plant Density	F = 23.97	P = <0.001
Interaction Effect			
	Hybrids X Plant Density	F = 7.54	P = <0.001
$LSD_{0.025}$	Hybrid		3.5
$LSD_{0.025}$	Plant Density		3.2
$LSD_{0.025}$	Hybrid X Plant Density		5.4
%CV			13.7

There was considerable variation in hybrid response to ear rot under different plant densities as shown in Table 5.1 and Figure 5.1. The differences between hybrids and plant densities, and the interaction between these two factors were all highly significant for the percentage diseased grain. From Table 5.1 it was apparent that few hybrids responded in the same way to ear rot colonisation under different plant densities. A hybrid such as PAN 6549 showed an increase in diseased grain with an increase in plant density. Most hybrids, such as PAN 6528, showed a bimodal increase in diseased grain at 36 000 and at 72 000 plants ha^{-1}. With these hybrids, the percentage diseased grain decreased at plant densities of 36 000 to 54 000 plants ha^{-1}. Other hybrids, such as PAN 6330, showed a rapid increase in diseased grain at plant densities of 18 000 to 36 000 plants ha^{-1}, and then a more gradual increase in diseased grain with increased plant density. Figure 5.1 showed the different types of hybrid responses to ear rot under different plant densities for the four selected hybrids.

From Table 5.1, it can be seen that there was considerable variation in the levels of diseased grain between hybrids. PAN 6549 and PAN 6429 had less ear rot overall than all other hybrids and PAN 394 had more diseased grain than all other hybrids. Overall, there was an increase in diseased grain at 36 000 plants ha^{-1} from 18 000 plants ha^{-1}, a reduction in diseased grain at 54 000 plants ha^{-1} from 36 000 plants ha^{-1}, and another increase in diseased grain from 54 000 to 72 000 plants ha^{-1}. However, the increase in diseased grain from 54 000 to 72 000 plants ha^{-1} was much smaller than the corresponding increase in diseased grain from 18 000 to 36 000 plants ha^{-1}.

All hybrids showed an increase in grain yield with a corresponding increase in plant density (Table 5.2 and Figure 5.2). However, not all hybrids responded in a similar manner to plant density. Some hybrids, such as PAN 6552, showed large increases in yield with increase plant density. Other hybrids, such as PAN 6528, showed a smaller gain in yield with increased plant density. These differences in grain yield response between hybrids resulted in a signifcant interaction response. Grain yield response at different plant densities can be seen for the selected hybrids in Figure 5.2.

Table 5.2: Grain yield (t ha^{-1}) for the various hybrids and plant densities during the 1986/87 season

	Plant density				
Hybrid	**18 000**	**36 000**	**54 000**	**72 000**	**Mean**
PAN 6330	5.99	8.00	8.94	9.06	8.00 a
PAN 394	6.30	8.97	9.25	9.29	8.45 ab
PAN 6429	6.80	9.34	9.46	9.48	8.77 b
PAN 6434	6.74	9.12	9.72	10.19	8.94 bc
PAN 6334	7.18	10.00	10.57	9.72	9.37 cd
PAN 6528	8.02	9.72	9.92	9.84	9.37 cd
PAN 6549	6.90	9.93	10.66	10.96	9.61 d
PAN 6557	7.45	10.16	11.00	10.67	9.82 d
PAN 6552	8.18	11.69	12.51	13.67	11.51 e
Mean	7.06 a	9.66 b	10.23 c	10.32 c	9.32

A summary of the ANOVA results.

Main Effect			
	Hybrids	F = 33.0	P = <0.001
	Plant Density	F = 171.85	P = <0.001
Interaction Effect			
	Hybrids X Plant Density	F = 1.85	P = 0.025
$LSD_{0.025}$	Hybrid		0.59
$LSD_{0.025}$	Plant Density		0.55
$LSD_{0.025}$	Hybrid X Plant Density		0.92
%CV			6.5

Overall, there was a increase in grain yield from 18 000 to 36 000 plants ha^{-1}, and again from 36 000 to 54 000 plants ha^{-1}. Most hybrids showed a grain yield plateau at 54 000 plants ha^{-1}. There was considearable variation in the mean yield over all plant densities. PAN 6330 (short season hybrid) had the lowest grain yield, whereas PAN 6552 had the highest mean grain yield.

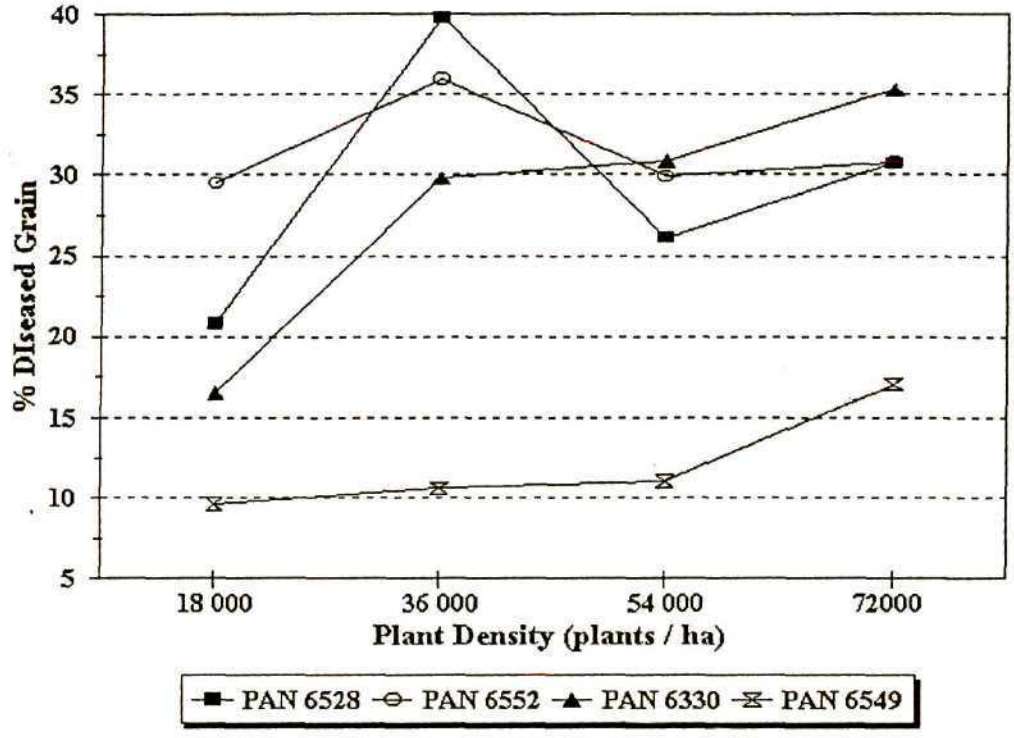

Figure 5.1: Percentage diseased grain for four hybrids at four different plant densities during the 1986/87 season.

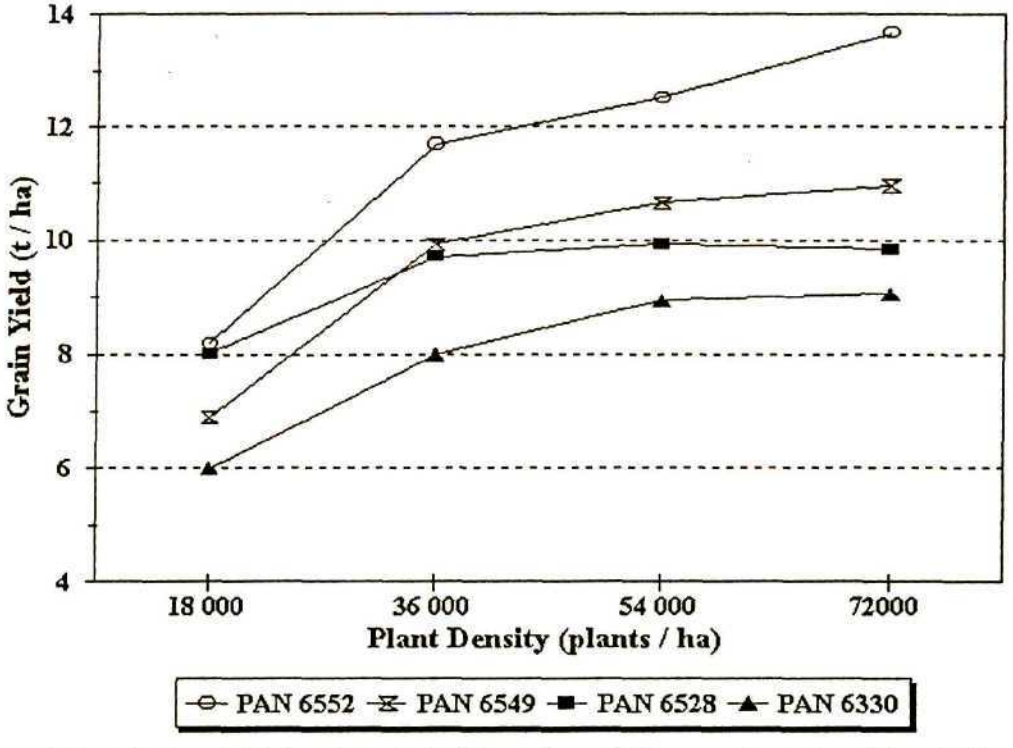

Figure 5.2: Grain yield for four hybrids at four different plant densities during the 1986/87 season.

Table 5.3: Correlation coefficients for prolificacy, shelling percentage, percentage grain moisture, percentage diseased grain and grain yield during the 1986/87 season

	Prol	Shell%	%Moist	DisGr	Yield
Prol	1.000				
Shell%	-0.220	1.000			
%Moist	-0.101	0.290 *	1.000		
DisGr	-0.169	-0.267 *	-0.364 *	1.000	
Yield	-0.499 *	-0.114	0.126	0.087	1.000

* = significant at the 99% level of significance.

Shell% = grain shelling percentage

%Moist = percentage grain moisture

DisGr = percentage diseased grain.

Prol = prolificacy

Yield = grain yield

The correlation matrix presented in Table 5.3 ($r^2 > 0.254$ is significant at the 99% level of significance) shows that the percentage diseased grain was negatively correlated with the shelling percentage and the grain moisture. Yield was negatively correlated with prolificacy.

1987/88 Experiments

Results for are presented in Tables 5.4 - 5.6 and Figure 5.3 and 5.4.

In this trial the number of plant densities was doubled and the number of hybrids increased from nine to twelve. Eight of the hybrids were common between the first two trials. From Table 5.4 it can be seen that the severity of infection in 1987/88 was lower than in 1986/87 and the variation in disease levels was higher. The variation in diseased grain between plant densities within a hybrid was also reduced compared to the previous season. Although not significant, the general trend across plant densities was for diseased grain to increase with increased plant density (Figure 5.3). Overall the mean percentage diseased grain for the densities of 18 000, 26 000, 34 000, 42 000 and 50 000 plants ha^{-1} were not significantly different from each other. Plant densities of 58 000 and 74 000 plants ha^{-1} had more diseased grain than all other plant densities but were not significantly different from the diseased grain at 42 000 and 66 000 plants ha^{-1}.

There was considerable variation between genotypes in the amount of diseased grain across all

plant densities. PAN 6549 had consistently less diseased grain over all plant densities, whereas PAN 6552 had more diseased grain over all plant densities. No interactions between plant density and hybrids were evident.

PAN 6552 had significantly more diseased grain than all other hybrids across all plant densities.

Table 5.4: Percentage diseased grain for the various hybrids and plant densities during the 1987/88 season

	Plant density								
Hybrid	18 000	26 000	34 000	42 000	50 000	58 000	66 000	74 000	Mean
PAN 6429	9.1	9.1	5.6	5.7	8.1	10.7	5.3	9.5	7.9 a
PAN 6549	8.3	8.4	12.0	7.6	9.9	13.3	10.4	13.4	10.4 ab
PAN 6363	7.7	12.5	12.8	9.5	10.7	13.8	15.1	12.3	11.8 bc
PAN 6514	11.1	16.1	12.2	9.6	10.2	13.6	16.2	14.9	13.0 bcd
PAN 6428	13.1	13.9	13.3	9.1	11.4	18.0	16.2	16.5	13.9 cd
PAN 6434	15.2	12.5	13.8	17.0	11.6	15.5	14.1	17.0	14.6 cd
PAN 473	8.7	9.6	25.7	14.5	10.7	14.2	18.2	17.4	14.9 cd
PAN 6334	10.2	14.4	11.8	14.8	17.8	20.0	16.2	23.1	16.0 de
PAN 6528	16.9	22.7	14.0	19.8	20.3	16.7	16.4	21.4	18.5 ef
PAN 394	20.2	18.9	13.1	22.6	24.9	24.2	19.0	17.8	20.1 fg
PAN 6330	20.1	14.7	19.3	25.5	17.3	25.7	26.1	28.2	22.1 g
PAN 6552	20.6	16.7	21.5	27.5	25.4	31.1	37.7	33.5	26.8 h
Mean	13.4 a	14.1 a	14.6 a	15.3 abc	14.8 ab	18.1 c	17.6 bc	18.8 c	15.8

A summary of the ANOVA results.			
Main Effect			
	Hybrids	F = 20.15	P = <0.001
	Plant Density	F = 4.36	P = <0.001
Interaction Effect			
	Hybrids X Plant Density	F = 0.304	P = *n.s.*
$LSD_{0.025}$	Hybrid		3.1
$LSD_{0.025}$	Plant Density		2.8
%CV			36.4

Table 5.5: Grain yield (t ha^{-1}) for the various hybrids and plant densities during the 1987/88 season

	Plant density								
Hybrid	18 000	26 000	34 000	42 000	50 000	58 000	66 000	74 000	Mean
PAN 6330	5.97	6.57	7.43	7.97	7.73	7.83	8.43	7.73	7.46 a
PAN 394	5.33	6.93	8.00	8.10	7.90	8.23	9.37	9.73	7.95 b
PAN 473	5.97	7.67	8.33	8.87	8.97	8.27	9.10	9.17	8.29 bc
PAN 6434	6.67	7.30	8.43	8.37	9.23	9.63	9.33	9.87	8.60 cd
PAN 6334	7.23	8.07	8.55	8.47	8.70	9.13	9.73	9.57	8.68 cd
PAN 6429	6.80	7.90	8.83	9.30	8.80	9.27	9.97	9.77	8.83 de
PAN 6528	6.77	7.63	8.67	9.53	9.17	10.17	9.87	10.07	8.98 def
PAN 6363	6.70	8.63	9.00	9.00	9.77	10.10	9.53	10.43	9.15 efg
PAN 6514	6.77	7.67	8.30	9.33	10.70	10.17	10.87	10.77	9.32 fgh
PAN 6549	7.73	8.90	9.60	9.20	9.50	9.90	10.07	9.73	9.33 fgh
PAN 6428	6.80	7.70	9.03	10.17	10.43	10.70	10.60	11.50	9.62 hi
PAN 6552	8.27	8.80	9.13	10.03	10.00	10.40	11.53	11.10	9.91 i
Mean	6.75 a	7.81 b	8.61 c	9.03 d	9.24 de	9.48 e	9.87 f	9.95 f	8.84

A summary of the ANOVA results.

Main Effect			
	Hybrid	F = 21.00	P = <0.001
	Plant Density	F = 76.63	P = <0.001
Interaction Effect			
	Hybrids X Plant Density	F = 1.05	P = n.s.
$LSD_{0.025}$	Hybrid		0.40
$LSD_{0.025}$	Plant Density		0.36
%CV			8.5

The grain yield data in Table 5.5 show highly significant differences between hybrids and plant densities but no interaction effects. From these data it is clear that increased plant densities resulted in increased yields, with significant differences between plant densities at almost every plant density. The grain yield response resulting from increasing lower plant densities was highly significant for all hybrids. However, in this season a yield plateau effect was reached at 66 000 plants ha^{-1}. PAN 6330 and PAN 6552 showed a reduction in yield when plant density was increased from 66000 to 74000 plants ha^{-1}.

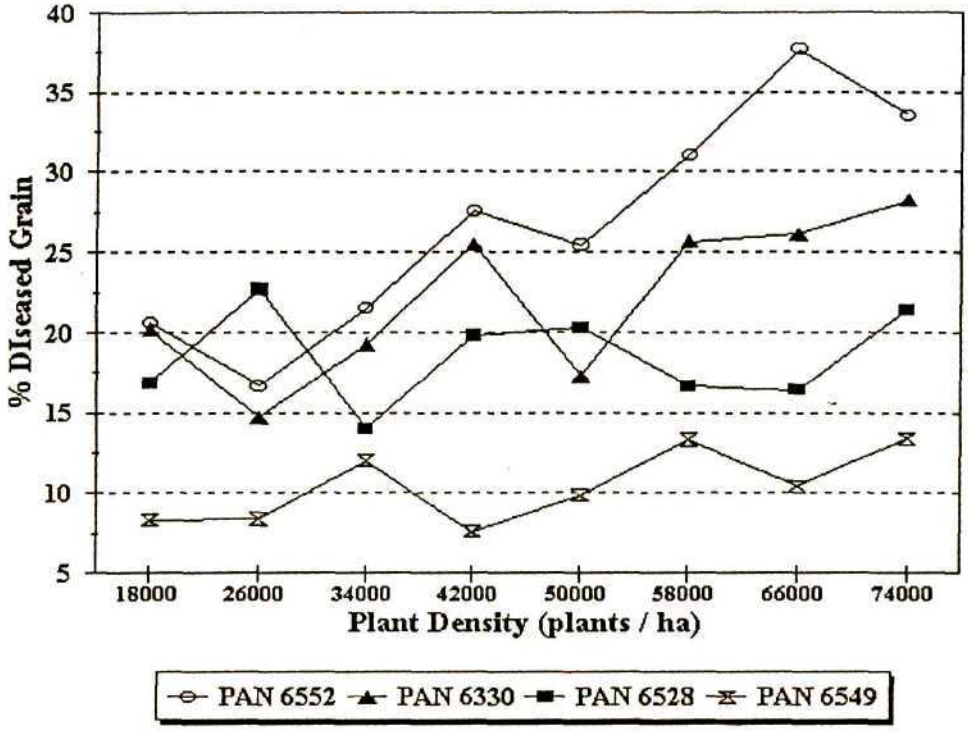

Figure 5.3: Percentage diseased grain for four hybrids at eight different plant densities during the 1987/88 season.

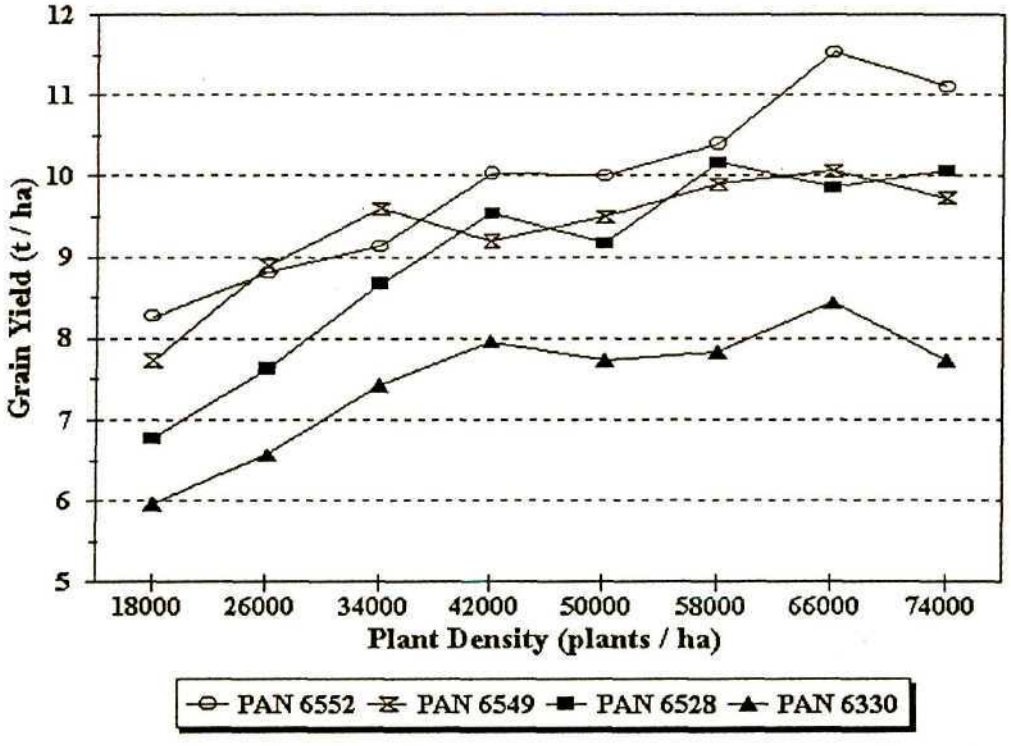

Figure 5.4: Grain yield for four hybrids at eight different plant densities during the 1987/88 season.

In Table 5.6 the significant correlations were the negative correlations between grain yield and prolificacy and the positive correlation between grain yield and percentage grain moisture. There was no correlation between the percentage diseased grain and any other factors.

Table 5.6: Correlation coefficients for percentage lodging, prolificacy, percentage diseased grain, percentage grain moisture and grain yield during the 1987/88 season

	Lodg	Prol	%Moist	DisGr	Yield
Lodg	1.000				
Prol	-0.249	1.000			
%Moist	0.092	-0.196	1.000		
DisGr	-0.249	-0.171	-0.096	1.000	
Yield	0.156	-0.463*	0.370*	0.030	1.000

Lodg = percentage diseased grain. Prol = prolificacy
%Moist = percentage grain moisture DisGr = grain shelling percentage
Yield = grain yield * = significant at the 99% level of significance.

1988/89 Experiments

Results are presented in Tables 5.7 and 5.8, and Figures 5.5 and 5.6.

Only differences in diseased grain between hybrids were significant. The interactions between hybrids and plant density were not significant. Table 5.7 shows that PNF 6459 was significantly more susceptible to ear rot than the other hybrids. Although there was variation in the levels of infection and colonisation between the other hybrids, the differences were not significant. No significant differences could be found between ear rot severity at the five different plant densities due to the high degree of variation. Figure 5.5 shows that the hybrid response to ear rot at the different plant densities was definitely not linear. The pattern of responses of the three hybrids with similar colonisation levels were not similar, especially at plant densities above 42 000 plant ha^{-1}. PNF 6459 had the highest percentage diseased grain when grown at 42 000 plants ha^{-1}.

Table 5.7: Percentage diseased grain for maize hybrids at various plant densities during the 1988/89 season

Hybrid	% Diseased Grain					
	34000	42000	50000	58000	66000	Mean
PNF 6459	19.35	22.65	13.50	14.75	16.80	17.41 b
PNF 6429	2.90	3.95	2.20	4.45	1.05	2.91 a
PNF 6552	6.30	2.65	4.80	7.90	5.55	5.44 a
PNF 6549	3.20	4.80	7.50	3.70	3.60	4.56 a
Mean	7.94	8.51	7.00	7.70	6.75	7.58

A summary of the ANOVA results.

Main Effect			
	Hybrids	F = 146.68	P = <0.001
	Plant Density	F = 3.56	P = n.s.
Interaction Effect			
	Hybrids X Plant Density	F = 2.90	P = 0.085
$LSD_{0.05}$	Hybrid		5.573
$LSD_{0.001}$	Plant Density		4.559
$LSD_{0.05}$	Hybrid X Plant Density		9.119
%CV			57.47

Table 5.8: Grain yield (t ha^{-1}) for maize hybrids at various plant densities during the 1988/89 season

Hybrid	Yield (Tonnes / ha)					
	34000	42000	50000	58000	66000	Mean
PNF 6459	8.490	9.130	9.330	9.275	9.795	9.204 d
PNF 6429	8.115	7.815	8.395	9.675	8.230	8.446 c
PNF 6552	4.170	4.745	4.770	5.290	5.060	4.807 a
PNF 6549	5.635	5.630	5.880	5.965	5.845	5.791 b
Mean	6.602 a	6.830 ab	7.094 abc	7.551 bc	7.233 bc	7.062

A summary of the ANOVA results.

Main Effect			
	Hybrids	F = 23.21	P = <0.001
	Plant Density	F = 2.10	P = 0.010
Interaction Effect			
	Hybrids X Plant Density	F = 0.71	P = n.s.
$LSD_{0.05}$	Hybrid		0.701
$LSD_{0.001}$	Plant Density		0.573
$LSD_{0.05}$	Hybrid X Plant Density		1.147
%CV			7.76

Table 5.8 shows that grain yield generally increased with increased plant density for all hybrids. There was a plateau effect in the grain yield at plant densities above 50 000 plant ha^{-1}. There were differences in yield potential between all hybrids. Based on the pedigree of PNF 6552, it would be expected to have the lowest yield potential due to a significant relationship between the two inbreds.

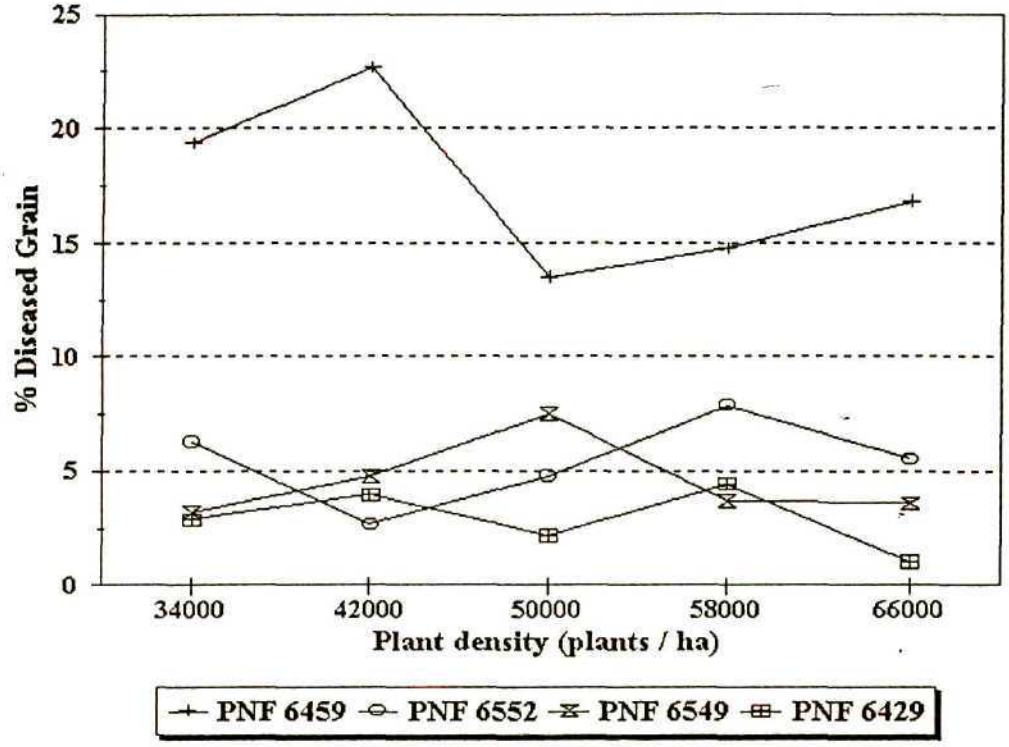

Figure 5.5: Percentage diseased grain for four hybrids at five different plant densities during the 1988/89 season.

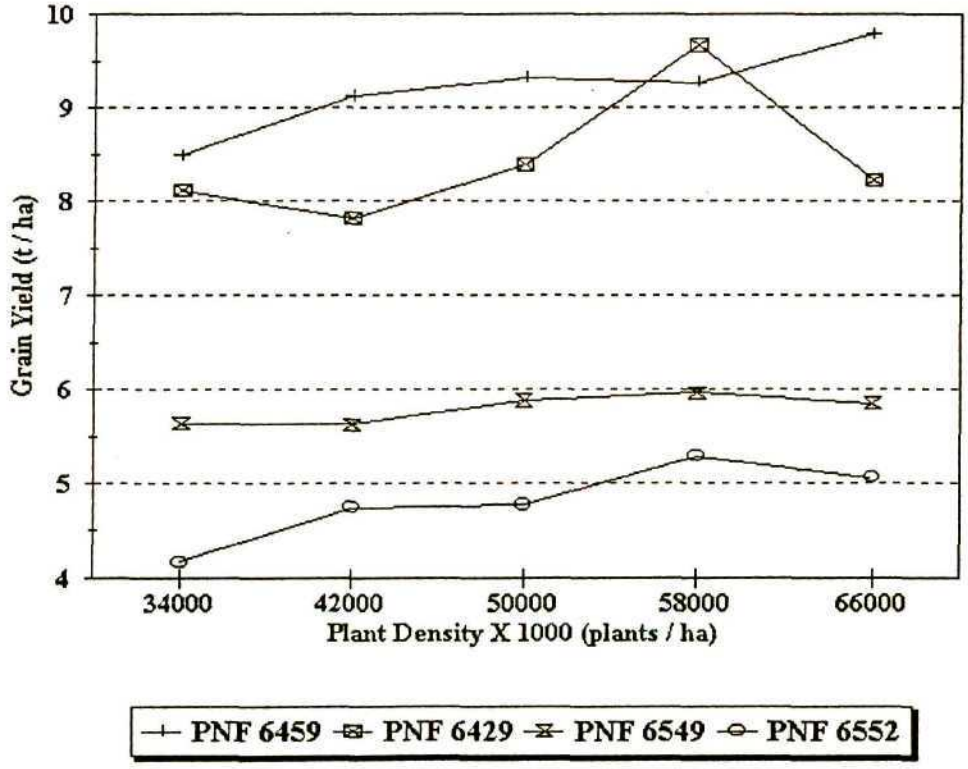

Figure 5.6: Grain yield for four hybrids at five different plant densities during the 1988/89 season.

5.4 DISCUSSION

In two out of three seasons there were highly significant differences between hybrids in their overall levels of diseased grain. This would be expected as there is considerable variation in the genetic composition of these hybrids. In all seasons the effect of plant density on the percentage diseased grain was highly significant. However, the interactions between hybrids and plant density were only significant in 1986/87 when the level of the percentage diseased grain was very high. The most likely reason is the very high co-efficient of variation in this trial. When it came to the final analysis of these data, it was not possible to re-analyse this trial with transformed data as the raw data had been lost due to computer failure.

The grain yield showed a similar response to that obtained for the percentage diseased grain. However, the major difference was that in all seasons the co-efficient of variation was very low. This difference between the co-efficients of variation for the percentage diseased grain and grain yield is usually the norm rather than the exception. A possible explanation for this is that disease, especially ear rot, is far more sensitive to variation in the environmental conditions and more likely to give variation within a trial than is grain yield. Experience has shown that conditions have to be near-ideal before an acceptable co-efficient of variation is obtained for accurate ear rot determination. As a result, the relative differences between hybrids are usually larger for grain yield than for the corresponding differences for the diseased grain.

From the above data it is clear that, for percentage diseased grain, the hybrids responded differently to the various plant densities in the three seasons. In 1986/87, hybrid responses fell into three distinct response group. There was an increase in diseased grain in a number of the yellow-grained hybrids at 36 000 plants ha^{-1} compared to 18 000 and 54 000 plants ha^{-1}. If these data are linearised, then the trend is for an increase in diseased grain with a corresponding increase in plant density. However, information is lost this way. Although the data in 1987/88 showed more variability, the overall trend is for an increase in diseased grain with an increase in plant density, especially above 50 000 plants ha^{-1}. Below 50 000 plants ha^{-1} the response was very variable. The white-grained hybrid, PAN 6429 and PAN 6549, was much more resistant to ear rot than the other hybrids. PAN 6528 showed a sharp peak in diseased grain at 26 000 plants ha^{-1}. PAN 6330 showed a significant increase in diseased grain at 42 000 plants ha^{-1} in 1987/88, which contrasts with the lack of correlation between these two factors in the 1986/87 season.

The trend for an increase in diseased grain for maize planted at densities greater than 50 000 plants ha^{-1} correlates with information from the USA (Koehler, 1959), where the majority of the maize is planted at plant densities of above 48 000 plants ha^{-1} (Aldrich *et al.*, 1975).

In contrast to the diseased grain information, the grain yield data showed little variance between hybrids. There was a sharp increase in grain yield when plant densities increased from 18 000 to 36 000 plants ha^{-1} (1986/87) and from 36 000 to 42 000 plants ha^{-1} (1987/88), before reaching a period of more gradual grain yield increases with increasing plant densities. Finally, a plateau in grain yield increase was usually experienced at around 54 000 plants ha^{-1}. A yield decrease was shown by PAN 6330, PAN 6549 and PAN 6552 when plant densities were increased from 58 000 plants ha^{-1} to 66 000 plants ha^{-1} in 1987/88. This would be expected as 1987/88 was a season a lower than expected rainfall, especially later in the growing season.

The type and range of hybrid response to ear rot exhibited by PNF 6459 (Figure 5.5) in 1988/89 was very similar to those exhibited by PAN 6528 and PAN 6552 in 1986/87 (Figure 5.1). The other hybrids in 1988/89 had a relatively low ear rot severity and their ear rot response were distinct from PNF 6459. PNF 6429 and PNF 6552 appeared to have two peaks in ear rot severity as measured by percentage diseased grain. PNF 6429 had higher levels of ear rot at 42 000 and 58 000 plants ha^{-1} than at 50 000 plants ha^{-1}. PNF 6552 had higher levels of ear rot at 34 000 and 58 000 plants ha^{-1} than at 50 000 plants ha^{-1}. Although this trend is small, a similar trend is evident in Figure 5.3 for PAN 6552 and PAN 6528 in 1987/88.

The increase in prolificacy of hybrids at low plant populations, compared to high plant populations, is significant. It was expected that there could be a correlation between the prolificacy index and the percentage disease grain due to the increased assimilate sink that was created with an increase in the number of ears per plant and hence grain yield. The greater sink could have predisposed the plants to ear rot pathogens (Dodd, 1980a and 1980b). This was not borne out in the correlations in Table 5.3 or Table 5.6. In fact, there was little correlation between any of the factors measured.

The rainfall distribution played a significant role in ear rot development during these three seasons. During the 1986/87 season, the rainfall during February was relatively high (the maize flowered in early February) followed by a some moisture stress in March and then good rains in April. The combination of periods of good rain with a little moisture stress at the start of

grain fill provided ideal conditions for ear rot infection and development. The weather during flowering and grain fill in 1987/88 was considerably drier and less conducive to ear rot than the previous season. Although the total rainfall for 1988/89 was significantly less than that of the previous two seasons, the rainfall for January and March was relatively high. However, the dry months of February and April resulted in conditions being less than ideal for ear rot development, and ear rot only developed on the most susceptible hybrid. It was this variation in climate that affected hybrid response to ear rot at different plant densities and resulted in an inconsistent response over seasons.

The parallel trends of ear rot severity, as measured by percentage diseased grain, as a function of plant density and hybrids observed in the first and third seasons suggest that there are distinct factors playing a role in ear rot development. Furthermore, this relationship cannot be explained solely by *S. maydis* infection and colonisation being a result of a source / sink relationship (Dodd, 1980a and 1980b). It would be easier to explain the response in terms of interactions between microclimate, conidium concentration in the atmosphere (Flett and Wehner [1989] showed that *S. maydis* conidia could be airborne) and canopy density. Low plant densities would allow easy spore penetration of the crop canopy (airborne of splash dispersal) for deposition onto maize leaves for infection, but the microclimate would not necessarily be ideal for infection and subsequent disease development. High ear rot incidences at low populations would tend to occur during seasons of higher rainfall at anthesis, grain fill and maturity. The effect of dry weather post-anthesis would have a marked effect on the incidence and severity of ear rot at these lower plant densities. At approximately 50 000 plants ha^{-1}, neither the microclimate nor the canopy cover is ideal for the pathogen, in terms of spore penetration of the canopy, and subsequent infection and disease development. At higher plant densities, the microclimate is ideal for infection but the plant canopy is dense and does not allow sufficient spore penetration into the canopy to result in severe epidemics. This would be similar to the effect found for grey leaf spot of maize (de Nazareno *et al.*, 1993; Rivera-Canales, 1993). A source / sink relationship could play a further confounding role.

Given the complexity of the interacting causes, it is proposed that further research under different environmental conditions, planting dates and inoculum pressure is needed to obtain a better understanding of hybrid responses to ear rot pathogens under different plant densities.

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CHAPTER 6

Breeding Strategies for Ear Rot Resistance in Maize Under Conservation Tillage [9]

ABSTRACT

The severe maize ear rot epidemics of 1986/87, 1987/88 and 1988/89 cropping seasons in South Africa forced maize breeders to reassess the importance of ear rot resistance, especially in the light of the prior lack of emphasis placed on this disease complex in their breeding programmes. The rapid and significant increase in the area of maize planted under conservation tillage, in association with maize monoculture, created an increased potential for ear rot epidemics. Breeders realised a need for increasing the inoculum pressure in their breeding programmes and to screen germplasm for ear rot resistance. It was no longer adequate to rely on natural ear rot inoculum to provide a satisfactory level of infection in order to select ear rot resistant material. Methods for artificial inoculation of breeding and screening nurseries were needed. The application of milled *Stenocarpella* infected ears, from the previous growing season, to the whorl of the plants about two weeks before anthesis, could be used to induce an ear rot epidemic (provided environmental conditions are conducive to ear rot infection and development). Breeding material which was very susceptible to the ear rot fungi needed to be either improved or eliminated from breeding programmes. The use of very susceptible inbreds in the production of commercial hybrids creates a risk to commercial crops being downgraded after harvest due to ear rot disease. The stability and level of ear rot resistance of commercial hybrids have received a relatively high priority in maize breeding programmes in South Africa.

6.1 INTRODUCTION

An increasing ear rot problem in maize (*Zea mays* L.) in South Africa, primarily caused by *Stenocarpella maydis* (Berk.) Sutton, was first recognised in the 1985/86 growing season in KwaZulu-Natal when there was an unexpected rise in the incidence of ear rots. Table 6.1 shows

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[9] This was an invited paper that has been rewritten since being published in the *Proceedings of the 9th. S.Afr. Maize Breeding Symp.* (1990), Tech. Comm. No. 232, Cedara 1988, H.O. Gevers (Ed.), Dept. Agric. and Water Supply, Pretoria, RSA. pp. 53-59.

the effects thereof on the downgrading of maize due to ear rot infection, primarily during the 1986/87, 1987/88 and 1988/89 growing seasons. This sudden and significant ear rot problem provided the incentive for maize breeders to re-assess the importance of resistance to the ear rotting fungi in their breeding programmes.

Contributing to this epidemic were a number of important factors such as climatic variation, conservation tillage, hybrid ear rot resistance and monoculture (Koster[10], pers. comm.; Farwell[11], unpublished; Nowell, unpublished). Correcting cultural practices and improving sanitation are short- to medium-term solutions to an ear rot problem, whereas breeding for ear rot resistance provides a medium- to long-term solution.

Table 6.1: Grade composition (%) of the annual South African maize crop from 1980/81 to 1993/94 (Viljoen[12], pers. comm.)

Marketing Season	Yellow Maize			White Maize		
	YM1	YM2	YM3	WM1	WM2	WM3
1984/85	87	13	0.5	86	12	1.5
1985/86	84	15	0.7	93	6	0.7
1986/87	67	31	1.7	89	10	1.0
1987/88	40	44	15.7	81	15	3.8
1988/89	43	51	6.2	66	28	6.1
1989/90	87	13	0.2	93	7	0.4
1990/91	73	25	1.2	88	10	2.0
1991/92	81	19	0.6	90	9	1.5

YM1 & WM1 = up to 4% by mass of discoloured and/or defective grain.
YM2 & WM2 = between 4 and 8% by mass of discoloured and/or defective grain.
YM3 & WM3 = greater than 8% by mass of discoloured and/or defective grain.

6.2 Conservation Tillage

Conservation tillage practices cause significant increases in the incidence of ear rot in South Africa and elsewhere (Kerr, 1965; Palti, 1981; Flett, 1990; Flett and Wehner, 1991; Flett *et al.*, 1992). Tables 6.2 and 6.3 show the significant increase in ear rots associated with reduced

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tillage at two sites in South Africa. The Geluksburg site is located in north-western KwaZulu-Natal and is in a relatively low rainfall region, while the Bloekomspruit site is about 40 kilometres east of Johannesburg in Mpumalanga (typical highveld region of South Africa).

Table 6.2 : The incidence of *Stenocarpella* spp. under different tillage systems at Geluksburg (Flett and Wehner, 1991)

Tillage	% Sten ears		% Rot kern.		% Smay kern.	
	*87/88	88/89	*87/88	88/89	*87/88	88/89
No Tillage	35.4a	8.5a	47.0a	20.8a	53.0a	20.7a
Chisel	15.3b	-----	45.8a	-----	27.1ab	-----
Chisel X2	13.7b	5.8b	37.9ab	15.7ab	25.8ab	10.9b
Chisel:Disc	-----	4,1b	-----	8.9b	-----	5.4b
Plough:Disc	7.8c	2.9b	32.4b	10.9b	11.4b	5.0b

* = log transformation
Means followed by the same letter are not significantly different at the 5% level (SNK test for significant differences)
% Sten ears = Percentage ears colonised by *Stenocarpella* spp.
% Rot kern. = Percentage rotten kernels determined by mass.
% Smay kern. = Percentage kernels colonised by *S. maydis* as determined in the laboratory.

The tillage treatments were as follows :

i) No tillage - the stalks were cut but the soil was left completely undisturbed.

ii) Chisel - the stalks were cut and then the soil was tilled using chisel tines.

iii) Chisel X2 - the above chisel process was repeated twice.

iv) Chisel:Disc - once the field had been chisel-tined, the field was disced to increase the incorporation of the maize debris in the top layer of soil.

v) Plough:Disc - after the stalks were cut, the field was ploughed to a depth of at least 700mm to ensure burial of the maize debris. The field was then disced to ensure a good seedbed for planting.

These tillage treatments gave a range in quantity of maize debris lying on the soil surface.

From Tables 6.2 and 6.3 it can be seen that the more debris that was left on the soil surface, the greater the prevalence of ear rots. It can also be seen that there were significant differences in the incidence of *Stenocarpella* spp. between the two sites. Visual assessment of the percentage diseased ears does not necessarily give a true reflection of the amount of diseased grain. This is due to concealed fungal infection that cannot be seen on the unshelled ear. Of concern was that the higher the colonisation rate, the greater the number of symptomless

colonised kernels, especially at Geluksburg.

Table 6.3 : The incidence of *Stenocarpella* spp. under different tillage systems at Bloekomspruit (Flett and Wehner, 1991)

Tillage	% Sten ears		% Rot kern.		% Smay kern.	
	*87/88	88/89	*87/88	88/89	*87/88	88/89
No Tillage	47.5a	20.3a	47.3a	21.5a	52.7a	25.3a
Chisel	45.9a	16.2a	39.6a	23.2a	47.3a	25.8a
Chisel:Disc	39.5a	8.8b	37.6a	18.6a	43.2a	27.2a
Plough:Disc	24.0b	5.0b	23.0b	7.0b	25.3b	6.3b

* = log transformation
Means followed by the same letter are not significantly different at the 5% level (SNK test for significant differences)
% Sten ears = Percentage ears colonised by *Stenocarpella* spp.
% Rot kern. = Percentage rotten kernels determined by mass.
% Smay kern. = Percentage kernels colonised by *S. maydis* as determined in the laboratory.

The area under conservation tillage is increasing rapidly in South Africa (Berry[13], pers. comm.) and in the light of the ear rot epidemic in the late 1980s, the importance of ear rot in maize breeding programmes had to be reconsidered. In order to do this, it was necessary to ensure high ear disease levels as well as good selection and breeding methods.

6.3 Artificial Epidemics

Given the effect of reduced tillage on increasing ear rots, inoculum pressure has to be considered in maize-breeding programmes. As the South African climate is so variable, natural epidemics do not occur with sufficient consistency or the infection levels are not high enough to ensure good selection pressure and to make accurate assessments of ear rot resistance of lines and hybrids over or within seasons. For this reason it is important that artificial epidemics be created to ensure adequate and uniform selection pressure. There are several ways that this can be done:

i) Improve conditions for infection and disease development through the use of irrigation
ii) Artificially introduce the pathogen **into** the plant to promote infection
iii) Artificially increase inoculum in the field and **on** the plant to increase opportunity for natural infection.

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The first method does not guarantee adequate infection, as the natural level of the inoculum in the environment may be very low, although conditions for infection may in other respects be ideal. The second method does not take mechanical "resistance" barriers into account, which can be significant (Koehler, 1959; Kerr, 1965), and some useful forms of resistance and/or avoidance may be discarded. The second method does not always give results that correlate well with resistance under natural epidemics (Rheeder and Marasas, 1994; Nowell, unpublished).

Inoculum can be introduced onto the plant and/or on the soil surface in a number of ways :

1) Practice monoculture, retaining as much plant debris on the soil surface as possible. Irrigation should also be available to ensure ideal conditions for infection and subsequent disease development.
2) Introduce colonised plant material (stalks or ears) into fields. Irrigation should also be available to ensure conditions are ideal for infection and subsequent disease development.
3) Introduce colonised plant material onto plants to promote infection. This can be done by introducing the fungi by means of naturally colonised material (such as milled *Stenocarpella*-infected ears from the previous season) or pure cultures grown on maize kernels specifically for this purpose. This material can then be placed in the whorl of the plant or at the base of the ear, although the latter may give rise to levels of infection that are too severe (Bensch, 1995; Nowell, unpublished).

The most natural and practical method of inoculation is (3), and hence it is the most suitable method for screening maize for ear rot resistance. Using this method, in association with irrigation, it is possible to control the quantity of inoculum (inoculum pressure), time of application, fungal species/biotype inoculated and free moisture once the inoculum has been applied (McLennan, 1989; Nowell, 1989; Flett and McLaren, 1994; Bensch, 1995; Farwell, unpublished; Nowell, unpublished). By applying inoculum to the soil surface and/or to the whorl of the plant at an early stage of plant growth, it is also possible to increase the amount of stalk rot (Warren and Shepherd, 1976; Nowell, 1989; Farwell, unpublished; Nowell, unpublished). Using this method, it is easy to apply inoculum rapidly to a relatively large number of plants in a short space of time, especially when compared to earlier methods. This allows for inoculation in the nurseries, at relatively low inoculum pressures, and for extensive screening of hybrids at higher inoculum pressures. Early inoculations using this method would allow all phases of the disease to be evaluated; e.g., the leaf / stalk / ear rot phases of

S. maydis, *S. macrospora* (Earle) Sutton and anthracnose (caused by *Colletotrichum graminicola* (Ces.) G. W. Wils).

Breeding for stable ear rot resistance should be advised by using durable or horizontal resistance. Horizontal resistance is a collective term for many resistance mechanisms that are continuously variable, and its inheritance is usually controlled by oligogenes. Breeding for horizontal resistance requires changes in the gene frequency of a population. In a horizontal resistance breeding programme:

a) There is no single good source of resistance but the breeding programme should be based upon a broad genetic base of relatively susceptible parents.
b) Vertical resistance must be eliminated initially from the parent populations as this form of resistance will mask horizontal resistance selection.
c) Population breeding techniques are used to increase the level of resistance.
d) The same single biotype of the pathogen should be used for screening for resistance, to help reduce the likelihood of vertical resistance selection.
e) Selection is for individuals that show low levels of disease (Robinson, 1987). This is difficult for ear rot resistance but the principles still apply.

Inoculum pressure should not be too high as this will mask many of the individual plants with useful levels of horizontal resistance. Once resistance levels have been built up to significant levels, inoculum pressure can be increased to test for ear rot resistance more thoroughly. Ultimately, it is important to test this resistance in as many locations and environments as possible in order to confirm the adequacy of the horizontal resistance developed.

6.4 RESISTANCE

A change in philosophy in maize disease resistance breeding was necessary in South Africa. In the past, resistance to tassel smut and northern corn leaf blight were bred into the commercial maize hybrids (Gevers, 1975a and 1975b; Gevers *et al.*, 1992). Once this task had been completed, commercial maize breeders placed less emphasis on diseases. A phenotypically balanced hybrid with a high grain yield potential and grain yield stability became the primary objective. During this period, maize ear rot become problematic as a result of an *S. maydis* inoculum build-up and favourable climatic conditions. The planting of highly ear rot susceptible maize on a large scale, and the increased use of reduced tillage, contributed greatly to the ear rot epidemics. Although grain yield potential and stability is of primary importance, selection

for disease resistance or the elimination of highly susceptible germplasm should continue unabated. Failure to do this will result in the introduction of ear rot susceptible hybrids on a large scale which would enhance the development of ear rot epidemics, should environmental conditions be conducive to ear rot.

Selecting for ear rot resistance is difficult for most commercial breeding programmes in South Africa, as these programmes are largely based in region were ear rot seldom is epidemic due to the hot and dry climatic conditions. For these reason, maize breeders, and their managers, need to develop effective strategies to breed for ear rot resistance or a least effectively screen their germplasm under local conditions.

Table 6.4 : Summary of ear rot incidence in six commercial hybrids from 1983 - 1986, expressed as a percentage of the mean percentage diseased grain for the trial

Hybrids	Season		
	1985/86	1984/85	1983/84
PAN 432	77.4	113.0	113.7
PAN 482	—	77.4	80.4
PAN 496	114.8	113.0	132.3
PAN 542	105.7	115.6	176.5
PAN 6514	93.3	89.5	81.0
PAN 6528	117.7	109.1	120.3
Mean Dis. Rating	1.5	1.3	1.4
No. Trials	9	7	7

Mean Dis. Rating = mean ear rot rating (1-9 linear scale) for the trial.

When breeding for ear rot resistance, it is usually found that resistance to the three main causal fungi, namely *S. maydis*, *S. macrospora* and *Fusarium graminearum* (Schwabe), is inherited independently (Koehler, 1959). There is also no correlation between stalk rot and ear rot resistance (Thompson *et al.*, 1971; Ooka and Kommedahl, 1977a and 1977b). However, Mesterhazy (1982) and Mesterhazy and Kovacs (1986) suggested that there may be correlation between the ear rot resistance to different ear rot fungal pathogens. There are large and consistent differences between genotypes (Hooker, 1956; Koehler, 1959; Sivasankar *et al.*, 1976; Jain *et al.*, 1981; King and Scott, 1981; Odiemah and Manninger, 1982; Gendloff *et al.*, 1986;

Ochor *et al.*, 1987). However, great variation in the incidence of the ear rots and resistance levels between years of specific germplasm has been found to occur in the USA (Thompson *et al.*, 1971). This is shown to be true for South Africa in Tables 6.4 (based an ear rot rating of diseased ears on a 0 - 5 scale, before ear rot became epidemic and a relatively low inoculum pressure) and Table 6.5 (based on the percentage diseased grain determined by mass during the seasons of ear rot epidemics and a relatively high inoculum pressure). These data for these tables was collected from a national series of yield trials and the ear rot was as the result of natural ear rot infection. These data showed that some South African commercial hybrids were highly susceptible to ear rot but hybrids with good ear rot resistance were also available.

Table 6.5 : Summary of ear rot incidence in nine commercial hybrids expressed as a percentage of the mean percentage diseased grain for the trial (1986-1989)

Hybrids	Season		
	1988/89	1987/88	1986/87
PAN 6462	82.2	77.0	92.3
PAN 6463	74.6	83.9	72.3
PAN 6549	106.9	77.1	80.4
PAN 6514	88.0	72.0	52.0
PAN 6528	100.7	119.8	107.0
AX 305 W	206.3	142.2	176.8
CRN 4502	115.4	122.2	181.6
RS 5206	54.1	85.3	75.7
SNK 2147	103.5	81.3	73.8
Mean Dis Rating	15.9	18.0	15.0
No. Trials	3	21	6

Mean Dis. Rating = mean diseased kernels for the trial, based on mass.

Koehler (1959) found that there was germplasm with superior ear rot resistance but this germplasm often did not have the necessary yield characteristics. Wiser *et al.* (1960) studied six inbreds, by artificially inoculating them with *S. maydis*, and found that ear rot resistance was quantitatively inherited, but partial dominance played a role. There was apparently no inbred that was completely resistant to the ear rot fungi, although there were high levels of resistance available (Koehler, 1959; Kerr, 1965). High-lysine maize was found to be hyper-susceptible to *S. maydis*, *G. zeae* and especially *F. moniliforme*. This trend could be traced from the

inbreds to the hybrids, but was dependent on the background of the material that the opaque-2 gene was introduced into (Ullstrup, 1971). In South Africa, high-lysine hybrids and inbreds have been developed that have effective levels of resistance to *S. maydis* ear rot (Gevers, 1989; Gevers *et al.*, 1992). In an analysis of *Stenocarpella* ear rot resistance, Gevers *et al.* (1992) found that there was effective levels of ear rot resistance throughout the inbred nurseries. There were significant differences in susceptibility between heterotic groups of inbreds, and between yellow grain, white grain and opaque-2 inbreds. Of the yellow grain inbreds, the heterotic groups F and M exhibited significantly more ear rot resistant inbreds than did other heterotic groups. The R heterotic group (Reid) showed a higher frequency of highly susceptible inbreds. The white grained inbreds exhibited a greater number of ear rot resistant inbreds than the yellow grained inbreds. The ear rot resistance was most frequent and highest in the F heterotic group. The opaque-2 maize inbreds showed a range in response to ear rot disease, with most showing resistance. A significant number of inbreds showed a high degree of resistance, particularly for the F and M heterotic groups. These trends could be seen in the ear rot severity of commercial maize hybrids in South Africa. B73-type parents, and to a lesser extent I137Tn, was blamed for the significant susceptibility to *Stenocarpella* ear rot in specific yellow grained commercial hybrids (Gevers *et al.*, 1992).

A diallel analysis of inbreds for ear rot resistance to *S. macrospora* by McLennan (1991), showed resistance to be mainly additive in nature, but dominance was significant at times. D940Y exhibited a high specific combining ability for ear rot resistance. Epistasis in resistance was also present in a number of inbreds. This meant that both recurrent selection and backcrossing could be used to improve *S. macrospora* ear rot resistance of susceptible inbreds, depending upon the resistance source (McLennan, 1991).

Heriditability of ear rot resistance is very complex, with many types of inheritance mechanism having been reported, which include additive resistance, dominance, modifier genes, epistasis and recessive resistance (Hooker, 1956; Wiser *et al.*, 1960; Boling and Grogan, 1965; Thompson *et al.*, 1971; Sivasankar *et al.*, 1976; Ooka and Kommendahl, 1977a and 1977b; Warren, 1978; McLennan, 1991; Gevers *et al.*, 1992). Most resistance mechanisms are additive in nature and that large genetic gains in resistance can be made in a relatively short period. The rate of progress in developing resistance to the ear rot complex and the heritability of the resistance will be significantly influenced by the base level of resistance of the germplasm and the intensity of the selection pressure (both inoculum pressure and quantitative or qualitative

selection), i.e. low levels of resistance can be masked by high disease pressure, particularly if selecting a small number of plants with the highest level of resistance in the given breeding source. The mode of inheritance of the ear rot resistance will determine the breeding methodology employed. It is important that breeders continue looking for new sources of ear rot resistant germplasm (usually in tropical germplasm) that can be adapted for local conditions and/or incorporated into locally adapted germplasm. Once the level of resistance in a breeding programme has reached adequate levels, it is important that screening pressure is maintained as quantitative resistance can easily be lost to stabilizing selection in the absence of ear rot selection pressure (Vanderplank, 1984).

When testers for ear rot resistance are selected, yield potential should be assessed too. Russell (1961) evaluated and discussed the various options available when testing maize for stalk rot resistance, and concluded that the tester should be able to reveal maximum genetic diversity and be a true test for stalk rot resistance. Russell concluded that testers with dominant or partially dominant resistance are not suitable testers for resistance as oligogenic resistance will be masked. A suitable tester would be an inbred, single cross or double cross, with strong additive resistance to ear rot while also being a suitable tester for yield. The same prinicples apply to ear rot resistance.

Significant differentiation between the response of hybrids to *Stenocarpella* ear rot infection and development, essentially that caused by *S. maydis*, has been demonstrated in South Africa. Although there is a complex interaction with the environment, differentiation is possible between hybrids. This allows for reliable recommendations to farmers regarding the relative susceptibility of hybrids to *Stenocarpella* ear rot infection. In general terms, most studies have shown that white-grained hybrids are more resistant to ear rot than yellow-grain hybrids (Rheeder, 1988; Gevers, 1989; Nowell, 1989 and 1992; Rheeder *et al.*, 1989; McLennan, 1991; Rheeder and Marasas, 1992 and 1994; Ferreira, 1994; Flett and McLaren, 1994; McLaren and Flett, 1994).

Rheeder (1988) and Rheeder and Marasas (1992) found significant differences between commercial hybrids in South Africa in their response to *S. macrospora*.

The ear rot fungi are only part of the whole stalk, root and ear rot complex (Koehler 1959 and 1960). Therefore, it is essential that the root and stalk rot phases are not ignored in breeding

programmes. The inoculation technique described above for ear rot, can also be used to induce stalk rot by inoculating the plants two to six weeks earlier.

6.5 Disease Assessment

Ear rot evaluation by differential apparent infection rates (Enerson and Hunter, 1980), is usually used for genetic studies and is very time consuming. Other ear rot assessment methods having been described (Hoppe and Holbert, 1936; Koehler, 1959; Pappellis *et al.*, 1973, Gulya *et al.*, 1980). Nowell (1989) and Nowell and Kaiser (1989) suggested that hybrid ear rot data be collected as the percentage visibly rotted kernels (by mass) and presented as a percentage rot relative to the mean of the trial.

Collection of ear rot data over several seasons and with multiple planting dates assists in making final evaluations of the relative susceptibility of hybrids. Ear rot data collected from multiple sites and seasons can be presented as a frequency table (the frequency being the number of times a hybrid was resistant or susceptible to ear rot), which will reflect the stability of the hybrid's resistance (Nowell, 1989). Due to the non-linear response of maize hybrids to ear rot disease under a variety of levels of inoculum pressure, ear rot data should be presented as a hybrid ear rot response pattern, also by using frequency tables (Nowell, 1989, 1992 and 1995; Nowell and Kaiser, 1989).

When making single ear selections, a few kernels can be removed from the base of the ear by hand in order to check whether or not there is any hidden *S. maydis*. In order to optimise accuracy and workload, the following system could be employed:

i) Preliminary ear rot screening could be undertaken by using a simple 0 - 5 rating scale based on the visual assessment of the ears.

ii) Ear rot data from the intermediate testing phase could be collected as the percentage of ears which have greater than 10 percentage of the ear rotted.

iii) The advanced testing phase usually needs to be accurate and could be based on the percentage of diseased grain.

The use of the different systems would be influenced by the need for accuracy, the amount of work involved and the practicality of the exercise (Nowell, 1989).

These methods are discussed in detail in Chapters 3 and 4. Flett and McLaren (1994) developed

a non-linear regression model to predict hybrid response to ear rot pathogens effectively, categorising hybrids into resistant / susceptible groups. The model can also be used to predict hybrid response under specific inoculum pressures. The model predicts that hybrids should be screened when disease levels for a trial are between 10 and 35% infected ears, and ideally between 17 and 20%. In trials with means either side of this range, it becomes difficult to accurately differentiate ear rot resistance levels between genotypes.

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SECTION II - GREY LEAF SPOT

CHAPTER 7

A Review of Grey Leaf Spot of Maize

ABSTRACT

Grey leaf spot (GLS) is caused by *Cercospora zeae-maydis* Tehon & Daniels. It is an aggressive fungal pathogen that is widely adapted and can severely damage maize foliage, reduce grain yield and increase the incidence of lodging, only recently has it been considered to be one of the most destructive maize diseases. The pathogen is associated with reduced tillage practices and, therefore, it has the potential to increase in importance worldwide, as reduced tillage becomes more widely implemented. The pathogen has been reported in the USA since 1924 and has occurred in epidemic proportions since 1974. Its control in the USA is based on rotation, tillage practices and planting of resistant hybrids. Although the pathogen has been studied for many years in the USA, much remains unknown about the fungus and the disease. Substantial research efforts are therefore needed in South Africa to understand the disease in the local context.

7.1 INTRODUCTION

Grey leaf spot (GLS), caused by *Cercospora zeae-maydis* Tehon & Daniels has become increasingly economically important in South Africa since 1992. This relatively new pathogen to South Africa has established itself primarily in the province of KwaZulu-Natal, and now causes significant yield losses each season (Ward *et al.*, 1993; Ward and Nowell, 1997). GLS was first identified by Tehon and Daniels (1925) in southern Illinois in the USA. Since the mid-1970s, this disease has become increasingly important in the USA and the distribution of *C. zeae-maydis* has increased significantly (Leonard, 1974; Roane *et al.*, 1974; Latterell and Rossi, 1983; Smith, 1988; Thorson and Martinson, 1993; Perkins *et al.*, 1995). Recent epidemics of GLS in both the USA and South Africa have resulted in *C. zeae-maydis* being recognised as a yield-reducing pathogen (Lipps, 1987; Smith, 1989; Lipps and Pratt, 1991; Rivera-Canales, 1993; Ward *et al.*, 1993; Gevers and Lake, 1994; Wegulo, 1994; Jenco, 1995; Ringer and Grybauskas, 1995; Ward and Nowell, 1997).

The aim in plant pathology is to arrive at a clear understanding the elements of each disease triangle or quadrangle studied (Robinson, 1976). The function of each contributing component studied is unimportant until integrated into the overall picture. This becomes difficult when a considerable amount of information is known about the disease. The concept of an ethograph (Putter, 1980) can be used to integrate and simplify information available on the disease quadrangle concerned. An ethograph is a graphical integration of epidemiological information. The ethograph starts from a central core of information and is built in a series of concentric spheres of information covering each systems level, from the molecular system in the middle to the population system on the outside. This concept has been taken and ethograph generated for *C. zeae-maydis* (Figure 7.1).

The net value of the ethograph is that it enables one to examine all contributing factors of a disease in one figure. The similarities and differences can be compared and identified. Based on the understanding on the key components of an ethograph, a series of intervention points can be identified at which disease control measures could be applied (Laing, 1987). The importance of each intervention point is related to the quantitative contribution of each factor in the epidemic.

This chapter follows the sequence as depicted in the ethograph in Figure 7.1.

1	No alternate hosts.
2	Insignificant teleomorph.
3	Long-distance wind dispersal.
4	Dormant phase after infection.
5	No free moisture required for infection.

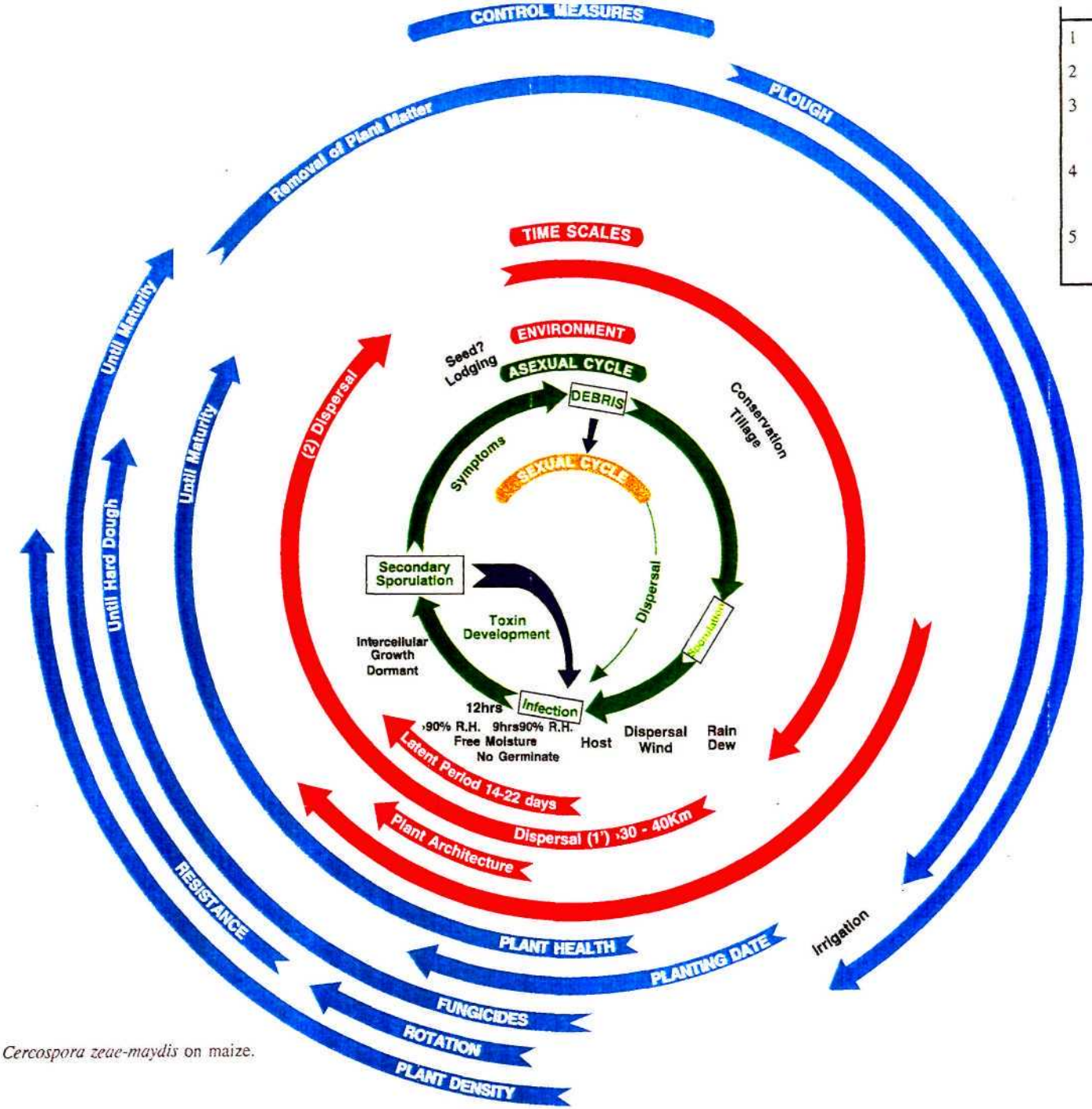

Figure 7.1 : Ethograph of *Cercospora zeae-maydis* on maize.

Since the first description of *C. zeae-maydis* by Tehon and Daniels (1925), a variety of descriptions of the fungus have appeared in the literature (Table 7.1).

Table 7.1: Summary of the morphological description of *C. zeae-maydis*

Conidium			Conidiophore		
Length (μm)	Width (μm)	Shape	Colour	Description	Author
50 - 85	5 - 9	hyaline, obclavate, 4- to 10-septa	olive - brown	single apical geniscar, 70 - 90 x 4μm, 3 - 8 septa	Tehon and Daniels, 1925.
30 - 90	5 - 9	hyaline, obclavate, 3- to 10-septa	olive - brown	occasionally 1 - 3 geniculate, 40 - 165 x 4 - 6μm, 3-- 8 septa	Chupp, 1953.
28 - 80	4 - 8	3- to 9-septa	brown	1 - 3 geniculate, 40 - 102 x 4μm, sparingly septate	Kingsland, 1963.
70 - 180	5-6 base & 2-3 tip	hyaline, 4 - 10 septa	dark	geniculate	Latterell and Rossi, 1983.

The teleomorph of *C. zeae-maydis* is a *Mycosphaerella* sp., which was found in overwintering field specimens by Latterell and Rossi (1983). The rarity of its occurrence suggested that it was not a significant source of inoculum in spring. There have also been no subsequent reports of the teleomorph.

Reports of *Cercospora sorghi* Ell. & Ev. being a causal agent of GLS of maize have not been substantiated as the descriptions published on *C. zeae-maydis* from maize are significantly different from those of *C. sorghi*. It is doubtful, therefore, that *C. sorghi* causes GLS on maize (Hyre, 1943; Mulder and Holliday, 1974; Shurtleff, 1980; Frederiksen, 1986).

In culture, *C. zeae-maydis* grows slowly and does not sporulate well. However, sporulation is improved by growing the fungus on V-8 juice agar or on decoction media made from green or senescent maize leaves (Beckman and Payne, 1983). Conidiophores are produced in both light and dark but a dark period is essential for the production of conidia (Latterell and Rossi, 1983).

Constant light inhibits germination, mycelial growth and sporulation. The optimum temperature range for growth was 22 - 30°C. Cultures can be stored for at least 24 months at 4°C on several different media (Beckman and Payne, 1983).

7.2 Crop Residue and Tillage Practices

C. zeae-maydis is a polycyclic, facultative pathogen (Chupp, 1953; Stromberg and Donahue, 1986) that overwinters in colonised maize debris (Beckman and Payne, 1982; Latterell and Rossi, 1983; Payne and Waldron, 1983; de Nazareno *et al.*, 1992). The increase in the incidence and severity of GLS over the last two decades has been linked with continuous maize production (Latterell and Rossi, 1983; Thorson and Martinson, 1993) and conservation tillage practices that leave substantial quantities of maize residue on the soil surface (Kingsland, 1963; Roane *et al.*, 1974; Hilty *et al.*, 1979; Beckman and Payne, 1982; Rupe *et al.*, 1982; Stromberg and Donahue, 1986; Payne *et al.*, 1987; de Nazareno *et al.*, 1992). Conservation tillage is described as any form of tillage that leaves at least 30% of the soil surface covered with crop debris. de Nazareno *et al.* (1993a and 1993b) found that the incidence of GLS increased with the amount of crop residue left on the soil surface, and that if crop residue covered more than 35% of the soil surface, GLS increased significantly. The influence of stubble has been demonstrated by Payne *et al.* (1987), who showed that colonised debris on the soil surface left by minimum tillage provided an earlier and more extensive source of inoculum than other tillage treatments. Grain yield from the no-till plots was significantly less than from the other plots. However, this trend could not be reproduced by de Nazareno *et al.* (1993a and 1993b). de Nazareno *et al.* (1993b) showed that a significant disease gradient occurred when inoculum sources were introduced into a field. The gradients were longer in the direction of the prevailing winds. Maximum infection or disease took place within six metres of the inoculum source. In the USA, government policies and economics favouring conservation tillage have led farmers to increase crop areas under such maize production practices, with the result that the incidence of GLS will probably remain high (Anderson, 1995). Whilst stubble tillage is recognised as a valuable tool for conserving soil moisture and reducing wind and water erosion, its beneficial effects may be offset by the increased primary inoculum levels of fungal pathogens such as *C. zeae-maydis* overwintering in the previous season's crop debris (de Nazareno *et al.*, 1990, 1992, 1993a and 1993b; Anderson, 1995).

Once established in a region, GLS often becomes a problem, even when conventional tillage is

used. The disease has become a problem in maize/soybean rotations in the USA where conventional tillage was practised (Perkins *et al.*, 1995). Similarly, Beckman and Payne (1983) found that in areas where inoculum had become abundant, this factor was more critical than other factors such as high humidity. In the USA, the disease is no longer limited to stubble tillage situations and the fungus has adapted to a wide range of cultural and environmental conditions. In most situations, the incidence of GLS will probably not greatly reduce yields, but given ideal environmental conditions, severe losses can result (Perkins *et al.*, 1995).

Smith (1989) found that there was an interaction between location and tillage effect. At one site, more severe GLS consistently occurred on the conventionally tilled areas than on reduced-till plots. However, at a second site, precisely the reverse occurred. Smith suggested that moisture stress had predisposed plants to infection by GLS, and plant stress levels were higher on the conventionally tilled plots than on reduced-till plots when moisture was in short supply. This tillage effect on the incidence of GLS was significant for GLS-susceptible hybrids but not for GLS-resistant hybrids. This finding has significant implications in the RSA, where drought stress is common.

Inoculum does not survive in leaf pieces for more than five months when buried 10 cm below the soil surface, whereas inoculum above the ground survives for at least six months (Ureta, 1985; de Nazareno *et al.*, 1992). Payne and Waldron (1983) found that the period of survival of buried inoculum varied between locations. Tillage operations resulting in the complete burial of debris have been demonstrated to be a means of managing GLS (Latterell and Rossi, 1983; Payne and Waldron, 1983; Stromberg, 1986; Spink and Lipps, 1987; Huff *et al.*, 1988; Ward *et al.*, 1993; Perkins *et al.*, 1995; Ward and Nowell, 1997). Discing provides insufficient burial of residues (Stromberg, 1986) and ploughing can leave as much as 10% crop residue on the land surface (de Nazareno *et al.*, 1993a and 1993b). This could provide sufficient inoculum to initiate an epidemic. Farmers cannot expect to control the disease by ploughing and burying infected debris in areas where there are abundant regional and external sources of inoculum (Smith, 1989). Such conditions are frequently encountered in South Africa (Ward *et al.*, 1993).

7.3 Crop Rotation and Sanitation

The pathogen does not survive much beyond a season in colonised debris and, because it is host-specific, rotation with other crops such as soybeans, dry beans and cereals is an alternative to

ploughing (Latterell and Rossi, 1983; Stromberg, 1986; Huff *et al.*, 1988). Sowing non-host crops for two years has been recommended where reduced tillage is practised in areas favourable for disease, or one year under clean ploughing (Spink and Lipps, 1987). Other pests and diseases of maize such as eyespot, ear- and root-rots are also increased under reduced tillage, making rotation an attractive option (Latterell and 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 (Channon and Farina, 1991; Ward *et al.*, 1993).

Harvesting maize for silage reduces the inoculum carry-over to the next crop since most of the foliage is removed before GLS becomes severe (Stromberg, 1986). Payne *et al.* (1987) found that lands where maize was cut for silage should be planted under conservation tillage practices, whilst lands that were harvested for grain could be ploughed to reduce the large quantities of infected debris left.

7.4 Hosts and Distribution

Stromberg and Donahue (1986) considered *C. zeae-maydis*'s host range to be limited to *Zea mays* L. However, McGee (1988) listed alternate hosts as being Barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.), Johsongrass (*Sorghum halepense* (L.) Pers.) and *Sorghum* spp. These are also considered to be alternate hosts of *C. sorghi* (Frederiksen, 1986). This is possibly due to *C. zeae-maydis* being confused with *C. sorghi*. *C. zeae-maydis* has not been recorded as being seed-borne and, therefore, is not considered to be seed-transmitted (McGee, 1988; Richardson, 1990). This is surprising as a number of *Cercospora* spp. are seedborne (Chupp, 1953; Neergaard, 1977; Richardson, 1990).

Grey leaf spot of maize remained obscure in the USA until the 1970s (Arnt, 1943; Hyre, 1943; Lehman, 1944; Graydon, 1963; Kingsland, 1963; Leonard, 1974; Roane *et al.*, 1974). The disease subsequently increased in the eastern states of the USA, especially in the more humid, mountainous areas, where it is associated with monoculture maize under reduced tillage practices (Leonard, 1974; Roane *et al.*, 1974; Hilty *et al.*, 1979; Latterell and Rossi, 1983; Lipps, 1987). The disease has since moved westward from the eastern states, and is now widespread in the mid-Atlantic and Midwest regions and continues to move westward into new ecological niches in the USA (Smith, 1988; Perkins *et al.*, 1995).

In his *Cercospora* monograph, Chupp (1953) identified those countries outside the USA where GLS occurred as Brazil, Colombia, Peru and Trinidad and has subsequently has been reported to occur in Brazil, Central America, China, Colombia, Mexico, South Africa, Trinidad, and Venezuela (Boothroyd, 1964; Latterell and Rossi, 1983; Ward *et al.*, 1993; Coates and White, 1994). In South Africa, GLS is well established in the province of KwaZulu-Natal and has been reported from other neighbouring provinces (Bensch and Flett, 1995; Ward and Nowell, 1997; Ward *et al.*, 1997).

7.5 INFECTION OF THE HOST AND DISEASE DEVELOPMENT

High humidity, suitable air temperature, host susceptibility and the presence of a source of inoculum are conditions necessary for a GLS epidemic (de Nazareno *et al.*, 1993b). Conidia are produced on colonised maize debris from the previous maize crop and in spring are carried by wind to infect a newly planted maize crop during moist periods.

Payne and Waldron (1983) found spore dispersal was at a maximum in the late afternoon. Leaf wetness of nine hours resulted in more than 90% conidial germination. A minimum of six hours was required for a significant proportion of the conidia to germinate. Non-germinated conidia were unable to survive wetting and drying but germinating conidia survived short dry periods without adverse effects on the germ tube. Optimum conditions for germination were reported to be 19 - 25°C and more than 95% relative humidity (Rupe *et al.*, 1982). Beckman and Payne (1982) induced conidia to germinate on plants after 24 hours at temperatures of 22 - 30°C, after the plants were exposed to high relative humidity by intermittent misting for a 12 hour period. Outside this temperature range, germination decreased. Optimal germination was recorded to occur under diurnal light. Germ tube growth occurred up to five days after inoculation. It was more extensive on the upper leaf surface in the presence of free moisture than in the absence of free moisture. Germ tubes may emerge from each cell of a conidium and grew for seven days or more on the lower leaf surface, where little or no free moisture accumulated. There was a positive tropism towards stomata under high relative humidity (Thorson, 1989; Thorson and Martinson, 1993). When the relative humidity was below 95%, germ tubes stopped growing but did not die. The germlings survived for at least six hours when the relative humidity was reduced to as low as 65%. When the relative humidity was increased to 95% again, the germ tubes resumed elongation. Numerous appressoria formed over stomata four to five days after germination, provided the relative humidity was high. A single conidium could give rise to as

many as eight appressoria over different stomata. In the presence of free water on the leaf surface, stomatal tropism was reduced and appressorial formation was rare which resulted in no penetration of the host tissue (Rupe *et al.*, 1982; Thorson and Martinson, 1993).

Unlike Beckman and Payne (1982), Gwinn *et al.* (1987) found that stomatal penetration (defined as the total stomatal penetration and not only those with appressorial formation) increased with plant age and there were small differences between genotypes. An infection peg penetrated the stoma 6 - 7 days after inoculation. Penetration only occurred from appressoria over stomata. At least 5% of the appressoria had resulted in penetration into the substomatal cavities 6 - 8 days after inoculation. An infection hypha usually develops a slightly enlarged, generally one-celled, sometimes two-celled, vesicle immediately after penetration. A robust primary hypha with septa grows from the vesicle until it encounters the parenchyma or mesophyll tissues (Beckman and Payne, 1982).

Stromberg (1986) also observed that colonisation of the leaf tissue was confined to the mesophyll and was intercellular. Delimitation of the hyphal growth lateral to the vascular system is by the sclerenchyma tissue surrounding major veins. This results in the typical long, narrow, parallel lesions.

Fungal stromata, which are formed in substomatal cavities, in the necrotic tissue, give rise to numerous conidiophores and conidia. The production of conidia usually commences 1-3 days after the lesion becomes necrotic. Necrosis of the cells is considered to be associated with toxin production and *C. zeae-maydis* only colonises cells once the tissue starts to deteriorate.

Latterell and Rossi (1983) found that a notable feature of *C. zeae-maydis* was its ability to survive adverse conditions once infection had taken place. Stromata in the substomatal cavities are able to survive dry periods and commence activity again to produce conidia after a brief exposure to moisture. Should the environmental conditions not be favourable for disease development, the fungus may remain dormant in the plant until conditions are favourable for further development (Stromberg, 1986).

Except for the need for high relative humidity, the environmental conditions required for GLS development remain vague. Ringer and Grybauskas (1995), in studies on infection cycle components of GLS disease progress, found that high levels of rainfall between planting and

infection, or rainfall during the primary sporulating period, may be more important than total seasonal rainfall. They concluded that rain in these early infection cycles may result in the generation of large quantities of inoculum, resulting in high disease levels in susceptible hybrids. Conversely, high levels of disease did not occur until late in the season when there were low levels of initial inoculum or a lack of rainfall during the period of early infection cycles. This appeared to be due to longer latent periods and a reduced number of infection cycles. Beckman *et al.* (1981) suggested high temperatures and lack of rainfall were not range- limiting factors for this fungus. Latterell and Rossi (1983) found that high levels of rainfall in spring did not ensure early and severe outbreaks of GLS, nor did a dry summer ensure low levels of damage. They concluded that a scarcity of rainfall and high temperatures (averaging 28°C) did not limit the development of GLS. Beckman and Payne (1982) suggested that the typical late-season appearance of GLS in the field is probably due to extended periods of high relative humidity, enhanced by the canopy effect of mature plants. Jenco (1995) suggested cumulative hours of relative humidity >90% could explain the GLS conidial concentration in the maize canopy air.

Field studies have shown that GLS frequently occurs after 12-13 hours of RH >90% and/or 11-13 hours of leaf wetness and such conditions usually occur two weeks prior to sharp increases in GLS incidence (Rupe *et al.*, 1982). In general, the pathogen requires periods of high relative humidity, ample free moisture, and cool, cloudy days for infection and disease development (Anderson, 1995). Such conditions frequently occur in mountain valleys and "river-bottoms" (Payne *et al.*, 1987), during overcast days, when mists extend the dew period (Latterell and Rossi, 1983), in close proximity to water bodies (Ayres *et al.*, 1985) and under overhead irrigation (Hawk *et al.*, 1985). These observations are supported by the finding that GLS increases in low lying areas (Spink and Lipps, 1987) and under overhead irrigation (Ward *et al.*, 1993). Furthermore, this pathogen can occur at relatively high elevations, which may be associated with mist belts in these regions (Latterell and Rossi, 1983).

7.6 Symptoms and Toxin Activity

Early signs of infection and colonisation are pin-point sized yellow flecks halo which are easily observed when the leaf is held to light. Chlorotic flecks are observed nine days after inoculation, and these elongate to form narrow lesion initials at 12 days. Characteristic mature lesions show after 14 - 21 days (Beckman and Payne, 1982; Ringer and Grybauskas, 1995). Ringer and Grybauskas (1995) established that latent periods (from inoculation to 50% sporulation) range

from 14 - 19 days for susceptible hybrids and 16 - 22 days for moderately resistant hybrids. Beckman and Payne (1982) found that young maize plants developed mature sporulating lesions 3 - 4 days earlier than did mature plants. On susceptible genotypes mature lesions are distinctly rectangular in shape, 10 - 70 mm long and 2 - 4 mm wide and are delineated by the veins on both sides of the lesion. They are tan to pale brown in colour and assume a grey caste during sporulation (Tehon and Daniels, 1925; Chupp, 1953; Latterell and Rossi, 1983; Ayres *et al.*, 1985; Stromberg, 1986). The diagnostic features of GLS lesions are the clear edges along major veins and the opacity of the mature lesion (Coates and White, 1994). The silhouette of the lesion, when held up to the light, is due to the formation of stromatic tissue and the dark, hardened mycelium of the fungus in the sub-stomatal cavities (Latterell and Rossi, 1983). This also allows for the detection of GLS, even when the host tissue has died, stomata showing on senesced leaf tissue as dark silhouettes (Latterell and Rossi, 1983; Coates and White, 1995).

Primary infections usually develop on the lower leaves and, when lesions mature, produce conidia that are able to germinate and infect adjacent leaves. As more lesions form, they may coalesce, and blighting occurs (Stromberg and Donahue, 1986). Extensive leaf blighting may develop until all leaves are killed (Stromberg and Donahue, 1986; Ward *et al.*, 1993; Ward and Nowell, 1997). Leaf sheath lesions occur in severely infected fields. Damage to stalks occurs when leaf blighting is severe and results in a high percentage of lodged plants (Stromberg and Donahue, 1986; Shurtleff and Pedersen, 1991). When leaf blighting by GLS is initiated early, resulting in significant blighting of the leaves during grain fill, stalk deterioration and increased lodging results (Roane *et al.*, 1974; Latterell and Rossi, 1983; Stromberg and Donahue, 1986). When there is greater demand for carbohydrates from stalks and root tissue by developing kernels as a result of decreased photosynthesis in diseased leaves, this pre-disposes the maize plants to stem- and root-rotting fungi and leads to increased lodging (Dodd, 1980a and 1980b). Severe lodging can adversely affect mechanical harvesting and results in further losses in grain yield due to a reduction in harvestable yield.

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 (Daub, 1982). The toxin is extremely toxic to plant cells, causing oxidation of fatty acids, sugars, cellulosic materials and amino acids, resulting in the destruction of cell membranes. Cercosporin acts as a photosensitising agent in the plant and is only able to kill plant cells in the light (Daub, 1982; Daub and Hangarter, 1983; Gwinn *et al.*, 1987). *C. zeae-maydis* has been shown to produce

cercosporin (Duvick, 1987). Tissue from older corn plants was less sensitive to cercosporin but varietal differences have not been observed (Gwinn *et al.*, 1987). There is considerable variation in the amount of cercosporin produced by various isolates of *Cercospora* which would result in different amounts of damage to the host cells (Jenns *et al.*, 1989).

7.7 Host Resistance

In the 1970s, when GLS was recognised as being a threat to maize production, efforts were made to find host resistance to the pathogen. Initially, all maize hybrids were reported to be susceptible (Roane *et al.*, 1974). Hilty *et al.* (1979) found little resistance in hybrids and only one inbred was found to have a high degree of resistance to GLS in Tennessee. Other researchers have reported that although the widely used inbreds B73, Pa91, Mo17 and A632 were found to be susceptible to GLS, the commercial inbreds B68, NC250, Pa875, Va14, Va17 and Va85 had high levels of resistance. More recent evaluations of commercial hybrids have shown that high yielding hybrids with good levels of resistance are available (Ayres *et al.*, 1985; Ureta, 1985; Roane and Donahue, 1986; Stromberg, 1986; Stuckey *et al.*, 1986; Hartman *et al.*, 1987; Johnson and Ayres, 1988; Stuckey *et al.*, 1988; Goodman *et al.*, 1989; Graham *et al.*, 1989; Johnson, 1989; Lipps and Pratt, 1989; Hartman *et al.*, 1990a and 1990b; Stromberg, 1990a and 1990b; Stromberg and Carter, 1991a and 1991b; Vincelli *et al.*, 1991 and 1994; Carter and Stromberg, 1992b and 1992c; Hawk and Weldekidan, 1992a and 1992b; Johnson, 1992; Saghai Maroof *et al.*, 1993 and 1996; Stromberg and Flinchum, 1993a, 1993c, 1994a, 1994b, 1995a, 1995b, 1996a and 1996b; Ward *et al.*, 1993; Coates and White, 1994; Gevers and Lake, 1994; Hohls *et al.*, 1995; Perkins *et al.* 1995; Lipps and Johnston, 1996; Ward and Nowell, 1997).

Host-resistance is now considered one of the best options for managing GLS, because there are good sources of resistance, and utilising resistance is simple for the farmer (Graham *et al.*, 1993; Coates and White, 1995). Hartman *et al.* (1990a) observed that a significant number of farmers in eastern Kentucky had moved from yellow- to white-grained maize because the white hybrids were more resistant to GLS. Donahue *et al.* (1991) found that existing commercial inbreds could be used to develop hybrids of acceptable yield and agronomic attributes with high levels of resistance to GLS. However, selection of a hybrid should not be based on GLS resistance alone, as the grain yield performance and standability of the hybrid are the most important factors (Smith, 1989; Ward *et al.*, 1993).

Maize breeding programmes aim, in addition to advances in grain yield and other agronomic traits, to increase the level of resistance to GLS in new hybrids. GLS resistance, as an additional criterion in breeding programmes, will reduce the amount of breeding material that meets the minimum performance level for yield and other traits in the programme, especially if selection pressure for GLS resistance is high (Anderson, 1995). The time-to-maturity of a hybrid is an important parameter to consider when breeding for GLS resistance. Long-season hybrids have potentially higher yields, but are at greater risk from GLS as they are subjected to blighting for a longer period during the grain-filling period (Stromberg and Donahue, 1986).

Most GLS resistance has been found to be quantitative in nature, with five or more genes involved (Ayres *et al.*, 1985; Thompson *et al.*, 1987; Huff *et al.*, 1988; Smith, 1989; Elwinger *et al.*, 1990; Ulrich *et al.*, 1990; Bubeck *et al.*, 1993; Anderson, 1995; Coates and White, 1995; Saghai Maroof *et al.*, 1996). However, there have been reports of resistance being inherited dominantly (Elwinger *et al.*, 1990; Gevers and Lake, 1994; Gevers *et al.*, 1994; Coates and White, 1995). Results of a diallel trial of twelve inbreds in South Africa suggested that resistance could best be defined by the additive-dominance model (Hohls *et al.*, 1995). Huff *et al.* (1988) undertook a complete diallel analysis of five inbreds and concluded that the inbreds Pa875, Va59 and B68Ht contributed significant levels of additive resistance to hybrids and that general combining ability (GCA) effects were 18 times higher than specific combining ability (SGA) effects. They also found an interaction between GCA effects and environment but the relative ranking of the inbreds changed only slightly.

In general, rate-reducing polygenic resistance acts by adding small increments of resistance which lead to an improvement in the level and stability of resistance. Conversely, major gene resistance, depending on a single gene, could be overcome by a single gene mutation in the pathogen and for this reason breeding for resistance on a polygenic basis is preferred (Latterell and Rossi, 1983; Ayres *et al.*, 1985). The variability of the fungus suggests that virulent strains would develop rapidly to overcome vertical resistance (VR) in maize if VR was introduced widely (Bair and Ayres, 1986). Ayres *et al.* (1985) suggested that resistance should be tested using more than one isolate of the pathogen through artificial inoculation in the field, in order to eliminate any differential interactions that many be present between the inbreds and isolates. At least two pathotypes should be sufficient to eliminate the differential interactions.

Interplot interference (or the cryptic error) in field experimentation can be problematic when

evaluating for resistance, resulting in an inaccurate measurement of a line or hybrid's resistance (Vanderplank, 1963; Robinson, 1976; Zadoks and Schein, 1979; Robinson, 1987). GLS is a pathogen that produces an abundance of airborne spores and there are large differences in susceptibility to the pathogen between hybrids and inbreds. Such material placed in a single, small plot trial could be significantly affected by interplot interference.

Coates and White (1994) undertook an extensive study of inbred resistance *per se* and found that of 396 inbreds tested, 343 inbreds showed lower levels of GLS than the susceptible check FR1141 and no inbreds were immune to GLS. It appeared that B68, B68Ht, T212, Va17 and Va59 did not have the same levels of resistance in Illinois as reported elsewhere (Coates and White, 1994). Gevers *et al.* (1994) found the inbreds KO54W and SO507W to have high levels of GLS resistance in South Africa. KO54W, in particular, was recommended because the resistance exhibited major dominance effects and could be used very successfully in backcrossing programmes. Even those inbreds with major additive effects showed some degree of dominance effects.

In a backcrossing programme for GLS resistance, Elwinger *et al.* (1990) found that the correlation between resistance in the source inbred and backcrossed inbreds was good. The best way of selecting material for such a programme was rather on the inbred *per se* than on the resistance of the inbred in test crosses. In specific cases, the resistance of the backcrosses differed significantly from the source line. Conflicting information regarding the dominance of GLS resistance in Va59, and some Pennsylvania inbreds, was evident but not all inbreds were common in all trials. Ulrich *et al.* (1990) showed that some inbreds (T222 and Mo18W) confer more resistance in crosses than they exhibit as lines *per se* and suggested both backcrossing and recurrent selection would be useful in improving GLS resistance levels in susceptible material.

Using restriction fragment length polymorphisms (RFLPs) and advanced statistical techniques, Bubeck *et al.* (1993) showed that specific quantitative trait loci (QTL) could be linked to GLS-resistance. However, these QTLs identifying GLS resistance were not consistent over locations. QTLs for GLS resistance differed for the three populations tested and only one region on Chromosome 2 in maize was associated with resistance in all populations. Saghai Maroof *et al.* (1996) have shown that certain QTLs are stable over locations and environments. This study showed that GLS resistance genes were present on Chromosomes 1,4 and 8 which explained 35-60%, 9-14% and 8-11% of the variance, respectively. Smaller QTL effects were noted on

Chromosomes 2 and 5 (although the Chromosome 5 gene effect was only present once). Except for Chromosome 4 (from the inbred B73), all resistance genes were derived from the GLS-resistant inbred Va14. Significant interaction occurred between Chromosomes 1 and 4. The fact that some of these QTLs are closely linked to other genes governing resistance to *Exserohilum turcicum* (Pass.) Leonard & Suggs, *Cochliobolus carbonum* Nelson and *Gibberella zeae* (Schw.) Petch., is important when using this technique in a breeding programme. Combining this technique with conventional breeding, it could be possible to pyramid the different genes in a specific background.

Hartman *et al.* (1990a) showed that composites and synthetics are available that show significant levels of GLS resistance. Graham *et al.* (1993) examined various selections of Iowa Stiff Stalk Synthetic and improved resistance through recurrent selection. They found that inbreds with acceptable agronomic qualities and significantly improved GLS resistance could be developed from this background. An elite group of lines showed increased GLS resistance, improved yield and improved standability. Further random mating of these elite lines and selection during inbreeding would further improve the GLS resistance and agronomic characteristics.

Moderately resistant hybrids were found to display chlorotic lesions (Roane *et al.*, 1974) and Ayres *et al.* (1985) found that resistance can be expressed as an initial chlorotic fleck, which later develops into a typical GLS lesion. However, some forms of rate-reducing resistance did not show chlorotic flecking. Susceptible inbreds generally display necrotic lesions (Huff *et al.*, 1988; Lipps and Pratt, 1989; Freppon *et al.*, 1994). Smith (1989) found a significant correlation between lesion length and final disease severity. Ringer and Grybauskas (1995) found that small differences in the number of lesions, latent period and sporulation contributed to significant, and sometimes large, differences between moderately resistant and susceptible hybrids.

A interesting phenomenon reported by Coates and White (1994) was that of variable lesion type within and between seasons. In this study, lesion types were divided into A = small chlorotic lesion, B = small restricted necrotic lesions with chlorotic halo, C = small rectangular lesion and D = large rectangular lesion. Some inbreds exhibited lesion types B and D, B and C, and C and D. Most of the variation occurred between seasons but it was possible to have different lesions types on the same plant.

Freppon *et al.* (1994) found a wide range of lesions types associated with inbreds, *per se*, or in

combination with other lines, with variable degrees of resistance. Pa875 possessed rate-reducing necrotic fleck resistance and was studied in combination with other inbreds of varying degrees of susceptibility. However, by the end of a season, these chlorotic flecks had developed into normal necrotic lesions. NC262A was found to have bright orange borders to the lesions, as had NC288. NC250A, on the other hand, had lesions surrounded by a yellow halo. The onset of necrotic lesions was either absent or delayed on both NC250A and NC288 and both produced restricted chlorotic lesions. Susceptible genotypes were characterised by the absence of chlorosis around lesions at all stages of development.

There is considerable variation in the rating methodology used to evaluate maize for the severity of GLS (Roane *et al.*, 1974; Hilty *et al.*, 1979; Rupe *et al.*, 1982; Ayres *et al.*, 1985; Stromberg and Donahue, 1986; Thompson *et al.*, 1987; Huff *et al.*, 1988; Lipps and Pratt, 1989 and 1991; Elwinger *et al.*, 1990; Ulrich *et al.*, 1990; Donahue *et al.*, 1991; Lipps and Johnston, 1996; Ward *et al.*, 1997). Thompson *et al.* (1987) and Elwinger *et al.* (1990) found that the assessment of GLS at any stage of the plant development, depending on onset of disease and hence disease severity, would result in similar relative rankings of the inbreds. The optimum time for a single disease assessment was shortly before the onset of natural senescence. Lipps and Pratt (1991) suggested rating the ear leaf only because this was found to give an accurate assessment of GLS response. In general, there is good correlation between the different rating methods. The growth stage at which a single GLS reading is taken can be very important because the later the reading is undertaken, the less chance there is of differentiation between certain genotypes. Saghai Maroof *et al.* (1993) proposed a simplified disease index rating system for its ease of use. The area under the disease progress curve (AUDPC - Berger, 1981) was readily calculated from the ratings over time. This method is sufficiently accurate to be used in association with restriction fragment length polymorphism (RFLP) resistance studies (Saghai Maroof *et al.*, 1993). Ward *et al.* (1997) showed that a whole plant, visual rating scale, was accurate in determining GLS severity in the RSA. This method was used extensively in evaluating GLS in fungicide and hybrid trials.

Thompson *et al.* (1987) and Elwinger *et al.* (1990) found that the assessment of GLS at any stage of the plant development, depending on onset of disease and hence disease severity, would result in similar relative rankings of the inbreds. The optimum time for a single disease assessment was shortly before the onset of natural senescence.

Natural infection is widely used to screen maize for resistance to GLS because an effective and efficient artificial inoculation technique is difficult to manage. However, it is possible to induce sporulation of the fungus on V8-juice agar, suspend the conidia in water and spray them onto the plants to create an epidemic. The major constraint is that environmental conditions have to be near-perfect for infection to take place (Thorson and Martinson, 1988; Jenco, 1995). Lipps and Johnston (1995 and 1996) showed that field inoculation with 20 - 50 *C. zeae-maydis* colonised oat kernels per whorl of each plant could result in an epidemic, provided a sufficient wet or high relative humidity period was provided through irrigation after inoculation. Conditions that simulated a dew chamber gave rise to significantly increased infections of maize leaves in the laboratory.

Greenhouse inoculations can be undertaken by spraying a conidial suspension ($2x10^4$ conidia ml^{-1} water) onto maize plants. A mister (a steamer can be used) must be run for a minimum of 12 hours to ensure optimum conditions for conidial germination and subsequent penetration of the stomata (Latterell and Rossi, 1983).

7.8 Chemical control

Research has been undertaken in the USA to determine the efficacy of fungicides and their economic feasibility in controlling GLS. Both protectant and systemic fungicides have been tested extensively (Hilty *et al.*, 1979; Ayres *et al.*, 1985; Smith, 1989; Lipps and Pratt, 1991; Stromberg and Carter, 1991c; Carter, 1992; Carter and Stromberg, 1992a and 1992d; Rivera-Canales, 1993; Ward *et al.*, 1993; Martinson *et al.*, 1994; Wegulo, 1994; Martinson and Munkvold, 1995; Ward and Nowell, 1997; Wegulo *et al.*, 1997). A summary of these results and comments is presented in Tables 6.1 and 6.3. These data show considerable variation.

The protectant fungicides mancozeb, maneb-zinc, chlorothalonil and copper thallate are only partially effective, especially under high inoculum pressure (Hilty *et al.*, 1979; Ayres *et al.*, 1985; Rivera-Canales, 1993; Martinson *et al.*, 1994; Wegulo, 1994; Martinson and Munkvold, 1995; Ward and Nowell, 1997; Wegulo *et al.*, 1997). The application of protectant fungicides, chlorothalonil and mancozeb, on a regular basis resulted in a significant reduction in the severity and incidence of GLS but often there was no corresponding significant increase in grain yield (Ayers *et al.*, 1985; Martinson and Munkvold, 1994; Wegulo, 1994; Wegulo *et al.*, 1997). In some trials, chlorothalonil was more effective in controlling GLS than propiconazole and

significantly increased the proportion of saleable seed in seed production fields (Rivera-Canales, 1993). When cost effectiveness was examined, the protectant fungicides were very competitive on nett profit per hectare. This was primarily due to the low cost of the protectant fungicides when compared to the systemic fungicides (Wegulo, 1994; Martinson and Munkvold, 1995; Wegulo *et al.*, 1997). Copper thallate, mancozeb and chlorothalonil are three protectant fungicides registered to control foliar pathogens of maize in the USA (Martinson and Munkvold, 1995).

Under conditions of drought stress it was found that chlorothalonil, at 5.03 kg a.i. ha^{-1}, could induce phytotoxicity. Copper thallate consistently resulted in phytotoxicity in the presence or absence of GLS, usually when more than one application per season were undertaken. The toxicity response was not hybrid-specific (Rivera-Canales, 1993; Martinson and Munkvold, 1995).

Trials in South Africa have shown benomyl to be highly effective in controlling GLS, with a single application delaying disease development for up to 40 days. The fungicide can also be applied aerially at 88 g ha^{-1} in a minimum of 40ℓ water ha^{-1} for maximum control. The first fungicide application should be when the lower leaves have not more than 5% leaf area loss due to GLS. The second application, if necessary, should be applied 21 - 30 days after the first application (Ward *et al.*, 1993; Ward and Nowell, 1997). These results confirmed extensive tests conducted in the USA with benomyl. Under light GLS pressure, no significant yield benefits could be found by applying benomyl (Smith, 1989). However, under high GLS pressure, yield increases ranged from 24 - 222% over the non-treated control. Associated with this was usually an increase in grain moisture at harvest as the plants stayed alive for longer. However, based on nett profit per hectare, benomyl was often less competitive than the protectant fungicides (Wegulo, 1994; Martinson and Munkvold, 1995; Wegulo *et al.*, 1997). Benomyl in combination with mancozeb was usually more effective than benomyl alone. When applied alone, neither benomyl or mancozeb had a significant effect on the growth or growth components of maize. However, together they significantly reduced the number of nodes (by one) per plant but did not have any negative effect on yield or other agronomic characteristics (Smith, 1989). Maneb-zinc also combined well with benomyl (Ayres *et al.*, 1985; Lipps and Pratt, 1991).

Table 7.1: A summary of yield increases (%) and comments associated with the research into the ability and economic feasibility of protectant fungicides to control GLS

Reference	Fungicide: Application Frequencies and Results			
	Mancozeb	Maneb-zinc	Chlorothalonil	Copper Thallate
Hilty *et al.*, 1979	27%			
Ayres *et al.*, 1985		Every 7 days = 20% Every 14 days = 8%	Every 7 days = 9% Every 14 days = 7%	
Smith, 1989	2%			
Rivera-Canales, 1993			12% yield increase 20% increase in proportion of all seed sizes (17% in small seed only)	Phytotoxic (4% - 10% lower yield)
Martinson *et al.*, 1994				1 appl. = significant increase but variable 2 appl. = phytotoxic
Wegulo, 1994	3 appl. = good control		5 appl. = good control	
Martinson and Munkvold, 1995	Crawfordville 1 appl. = 6% Crawfordville 2 appl. = 10% Conrad 1 appl. = 0% Conrad 2 appl. = 8%			
Wegulo *et al.*, 1997	Good cost benefit ratio for 3 applications = high returns		Good cost benefit ratio for 5 applications = high returns	Not economically viable Phytotoxic
Martinson, pers. comm.	11% more saleable seed (high disease)			

appl. = application/s

Table 7.2: A summary of yield increases (%) and comments associated with the research into the ability and economic feasibility of benzimidazole fungicides, alone and in combination with other fungicides, to control GLS

Reference	Fungicide: Application Frequencies and Results				
	B	B & M-Z	B & M	C & FZ	C & FT
Ayres *et al.*, 1985		7 days = 12% 14 days = 10%			
Smith, 1989	2%		6% Reduced nodes <1%		
Lipps and Pratt, 1991		Wooster = 11% Warsaw = 9% Not all hybrids equal			
Stromberg and Carter, 1991	2 appl. = 32% 4 appl. = 36%				
Carter, 1992	4 appl. = most effective				
Carter and Stromberg, 1992a	2 appl. = 114% 4 appl. = 122%				
Stromberg and Flinchum, 1993	2 appl. = 210% 4 appl. = 222%				
Ward *et al.*, 1993	1 application = up to 40 days control				
Ward and Nowell, 1997	Cedara 1992/93 = 73% 1993/94 = 44% Greytown 1992/93 = 24% 1993/94 = 60%			Cedara 1992/93 = 83% 1993/94 = 53% Greytown 1992/93 = 32% 1993/94 = 81%	Cedara 1992/93 = 79% 1993/94 = 61% Greytown 1992/93 = 15% 1993/94 = 76%

B = Benomyl B & M-Z = Benomyl and maneb-zinc B & M = Benomyl and mancozeb
C & FZ = Carbendazim and flusilazole C & FT = Carbendazim and flutriafol appl. = application

Table 7.3: A summary of yield increases (%) and comments associated with the research into the ability and economic feasibility of the triazole fungicides to control GLS

Reference	Fungicide: Application Frequencies and Results					
	Propiconazole	Tebuconazole	Flusilazole	Flutriafol	Difenoconozole	RII7592
Stromberg and Carter, 1991	2 appl. = 17% 4 appl. = 37%	2 appl. = 25% 4 appl. = 24% & reduced lodging	2 appl. = 23% 4 appl. = 30%			2 appl. = 30% 4 appl. = 28%
Carter and Stromberg, 1992a	2 appl. = 42% 4 appl. = 70%	2 appl. = 48% 4 appl. = 74%	2 appl. = 81% 4 appl. = 83%			2 appl. = 88% 4 appl. = 119%
Rivera-Canales, 1993	n.s. but increased					
Stromberg and Flinchum, 1993	2 appl. = 114% 4 appl. = 168%	1N 2 appl. = 77% 4 appl. = 133% 2N 2 appl. = 96% 4 appl. = 142%	2 appl. = 168% 4 appl. = 215%			2 appl. = 170% 4 appl. = 204% & grain moisture increase from 18% to 27%
Martinson, per. comm.	High dis. pressure = 18% increased saleable seed (seed size)					
Martinson and Munkvold, 1994	Crawf. 1 appl. = 28% 2 appl. = 14% Conrad 1 appl. = 13% 2 appl. = 14%					
Martinson *et al.*, 1994	Best = 2 appl. + 1 mancozeb					
Wegulo, 1994	Best = 2 appl. + 1 mancozeb					
Ward and Nowell, 1997				Cedara 1992/93 = 83% 1993/94 = 44% Greytown 92/93 = 43% 93/94 = 92%	Cedara 1992/93 = 83% 1993/94 = 48% Greytown 92/93 = 17% 93/94 = 74%	
Wegulo *et al.*, 1996	Good cost benefit ratio - variable. Generally one appl. most profitable					

appl. = application n.s. = non-significant Crawf. = Crawfordville 1N = normal concentration 2N = double normal concentration

One application of propiconazole usually results in an increased yield but there have been exceptions, when final GLS levels were over 20% (Martinson and Munkvold, 1995). From Table 6.3 it can be seen that the application of propiconazole usually increases yields between 13 - 168%. In addition, the amount of saleable seed was also increased in seed production fields. In some cases, the nett profit from a single application of propiconazole competed with the protectant fungicides (Stromberg and Carter, 1991c; Carter and Stromberg, 1992a; Rivera-Canales, 1993; Stromberg and Flinchum, 1993b; Martinson *et al.*, 1994; Wegulo, 1994; Martinson and Munkvold, 1995; Wegulo *et al.*, 1996; Martinson[14], pers. comm.). There are a number of other triazole fungicides that are very effective in reducing GLS and increasing yield from 17 - 170%. The cost effectiveness of all these products has not yet been determined (Stromberg and Carter, 1991c; Carter and Stromberg, 1992a and 1992d; Stromberg and Flinchum, 1993b; Ward and Nowell, 1997). Of the systemic fungicides tested, the triazole fungicides mixed with carbendazim resulted in the most effective control of GLS in South Africa. These products have now been registered for controlling GLS in both commercial and seed production maize fields in South Africa (Krause *et al.*, 1996; Ward and Nowell, 1997).

Chemical control measures in the USA are not widely recommended as these are usually uneconomical, except for seed producers (Ringer and Grybauskas, 1995). Many factors affect a decision to apply a fungicide to control GLS, the most important ones being:

i) the growth stage that the epidemic starts
ii) the germplasm planted (resistance level)
iii) the prevailing weather conditions.

Before using fungicides to control GLS, it is important to determine the economic feasibility of such an applications. In a USA study, net returns varied within and between seasons, being affected by climatic conditions, timing of fungicide application and the number of applications (Martinson *et al.*, 1994; Wegulo, 1994; Martinson and Munkvold, 1995; Wegulo *et al.*, 1997).

In the USA, propiconazole is the only systemic fungicide that is registered on maize (seed maize) for foliar disease control, including GLS control. Propiconazole cannot be applied after silking but mancozeb can be applied up to 40 days from harvest. In trials in Iowa in seed production fields, the application of propiconazole was of consistent economic benefit (Martinson and Munkvold, 1995). In order to maximise seed yields in Iowa, it is necessary to apply

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fungicides to control foliar diseases. The timing of the application of the fungicides is critical. The most cost-effective fungicide treatments tested for the control of GLS were five applications of chlorothalonil, three applications of mancozeb or two applications of propiconazole followed by one application of mancozeb. Both total grain and seed yield were improved significantly. As few as two applications of chlorothalonil resulted in significantly higher seed yields. Increased net returns were influenced by GLS severity, time of fungicide application, frequency of application and the genotype (Wegulo, 1994). Detasseled maize showed increased levels of GLS when compared to male sterile inbreds that were not detasseled, suggesting that detasseled maize may need extra protection (a fungicide application) to limit the effect on grain yield (Martinson *et al.*, 1994).

Martinson *et al.* (1994) reported trials in Iowa which were abandoned because of an interaction between fungicides and 2,4-D herbicide damage. This interaction resulted in damage to plants in these trials, confounding the results.

Bair and Ayres (1986) noted significant variability in the natural *C. zeae-maydis* isolates collected in the fields and speculated that this could mean that resistance to single--site systemic fungicides would arise relatively rapidly. This means that other control measures should be used whenever possible and if fungicides are used, then they should be applied judiciously. In particular, established fungicide-resistance management strategies should be implemented (Delp, 1988).

7.9 Other Factors

Many foliar diseases of maize develop when the crop moves into the reproductive phase of development, suggesting that they are low sugar diseases (Vanderplank, 1984; Robinson, 1987). Rupe *et al.* (1982) suggested plant maturity to be an important factor in GLS development as initial symptoms often appeared at anthesis. However, earlier research by Hilty *et al.* (1979) found that GLS was not necessarily associated with senescence as they produced GLS symptoms in the greenhouse on two- to three-week old seedlings. Beckman and 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.

Overhead irrigation, usually through a centre pivot, can be a significant factor in increasing the

incidence and severity of GLS. This should be taken into account when considering a management strategy to control GLS (Ward *et al.*, 1993).

Earlier findings suggested that high plant populations created high relative humidity microclimates favourable for disease (Beckman and Payne, 1983; Payne and Waldron, 1983; Ayers *et al.* 1985). However, Smith (1989), de Nazareno *et al.* (1991) and de Nazareno *et al.* (1993a and 1993b) proposed that less disease per plant occurs under high populations because of a "shielding" effect from spore interception in the denser canopies than in plots where canopies were open. Rivera-Canales (1993) supported this proposal, finding that GLS developed in seed crops when there was significant removal of leaf tissue during detasselling, and when the removal of male rows opened the canopy. Similar findings were reported by Carrera and Grybauskas (1992) but they found an interaction between planting date and plant density. Under conditions of high inoculum levels, the lower plant densities had significantly higher disease levels. No nitrogen effect was found in this trial but the less the shading, the more severe the disease. It was suggested that this may be directly linked to the effects of cercosporin in the light (Smith, 1989; Carrera and Grybauskas, 1992). Smith (1989) suggested that the relative conidial deposition rate may be increased at the lower plant densities due to reduced leaf area per unit ground area. If GLS is measured as disease per unit ground area, then there is more GLS at the higher plant densities. However, GLS measured per plant shows the opposite to be true.

Smith (1989) found that GLS increased significantly in severity with increasing levels of nitrogen fertilization in the ammonium nitrate form. In contrast, Carrera and Grybauskas (1992) tested various nitrogen levels at different plant densities, but observed no nitrogen effects on GLS severity. Smith (1989) found no significant reduction in GLS severity was observed in response to potassium fertilization but potassium fertilisation did result in increased total and green leaf area, and grain and silage yields. There was no GLS response to phosphate fertilization. Unknown variables were the original potassium and phosphate levels in the soil.

Weed control is important to increase air flow within the canopy, thereby reducing the relative humidity, to reduce the length of time conditions are favourable for infection (Spink and Lipps, 1987).

7.10 Yield Loss

Yield losses are correlated with the length of time that leaf-blighting is present during grain-fill: the longer the period of blighting before physiological maturity, the greater the yield losses. Early infection of GLS resulting in leaf blighting and premature death of the maize plant can seriously affect yield (Stromberg and Donahue, 1986; Nutter *et al.*, 1995). Hilty *et al.* (1979), Latterell and Rossi (1983), Ayres *et al.* (1985), Smith (1989) and Jenco (1995) showed that this disease can cause grain yield losses of up to 79%. However, grain yield losses are usually from 0 - 30% (Hilty *et al.*; 1979; Latterell and Rossi, 1983; Ayres *et al.*, 1985; Donahue *et al.*, 1991; Lipps and Pratt, 1991; Martinson *et al.*, 1994; Wegulo, 1994; Jenco, 1995). Stromberg and Donahue (1986) showed that for each one unit change in a 1 - 5 disease index, late maturing hybrids lost 1.06 tonnes ha^{-1} and lodging increased by 12.4%, whereas mid-season hybrids lost 0.70 tonnes/ha and lodging increased by 4.0%, and early maturing hybrids decreased in yield by 0.10 tonnes ha^{-1} and lodging increased by 7.5%. Shurtleff and Pedersen (1991) reported losses due to stalk deterioration after severe *C. zeae-maydis* infection of up to 100% of the leaf area in 1973 and 1974 in Virginia. Similar losses have been found by other researchers (Roane *et al.*, 1974; Latterell and Rossi, 1983; Smith, 1989). In South Africa, grain yield losses range from 0 - 50% (Ward *et al.*, 1993; Ward and Nowell, 1997).

In hybrid seed production in Iowa, USA, it was found that the total grain yield and the saleable seed fraction was significantly reduced by *C. zeae-maydis* infection (Rivera-Canales, 1993; Jenco, 1995). Jenco (1995) found that the most reliable estimate of yield loss incurred by GLS was a critical point estimate of GLS at the soft dough stage but that the AUDPC values could be used in epidemics that were severe or early. For AUDPC values, it is necessary for precise and reliable measurements of GLS to be taken regularly. When GLS was measured in the middle and upper canopy, the results were closely correlated with the yield losses measured.

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CHAPTER 8

Economic Losses as a Result of Grey Leaf Spot on Maize in KwaZulu-Natal, South Africa

ABSTRACT

Grey leaf spot (GLS) is a disease of increasing importance on maize in some of the major maize-producing regions of the world. It is highly aggressive pathogen, adaptable and severely affects maize foliage, reducing grain yield and increasing the incidence of lodging. It is therefore considered one of the most destructive of the maize diseases. Although the pathogen has been studied for a number of years by researchers in the USA, there are still many unknowns about the fungus and the disease in South Africa. Significant research efforts are therefore needed in South Africa to understand the disease in the local context. This study was initiated to accurately determine the grain yield loss due to GLS in the KwaZulu-Natal region.

In trials conducted at Cedara and Greytown during the 1992/93 and 1993/94 seasons, GLS severity was greater at Cedara than at Greytown in both seasons. Variation in grain yield loss was as high between locations within a season as between locations over two seasons. Grain yield losses in fungicide trials at Cedara and Greytown were 38 - 45% and 30 - 48%, respectively. Economic losses were R1 919 - R2 278 ha^{-1} at Cedara and R1 554 - R1 726 ha^{-1} at Greytown. The increase in GLS on reduced tillage treatments did not reduce yield when compared to conventional tillage. During the dry 1992/93 season, the reduced till treatments yielded significantly higher than the conventionally tilled treatments. The trial conducted on 49 hybrids confirmed that there was a larger yield loss due to GLS at Cedara than at Greytown (conventional tillage at both sites) but seasonal variation was larger than variation between sites. Hybrids were grouped into a susceptible group and a more resistant group based on GLS severity over both seasons. Overall, susceptible hybrids lost 37% (3.840 t ha^{-1}) in grain yield and resistant hybrids lost 5% (0.517 t ha^{-1}) in grain yield. Predicted hybrid grain yield losses ranged from 13% - 37% (1.286 - 4.032 t ha^{-1}) or R836 - R2 621 ha^{-1}, depending upon the level of inherent GLS resistance.

8.1 INTRODUCTION

Grey leaf spot (GLS), caused by *Cercospora zeae-maydis* Tehon & Daniels, has become increasingly important in South Africa since 1989. This relatively new pathogen to South Africa has since 1989 established itself, primarily in the province of KwaZulu-Natal, and now causes significant yield losses each season (Ward *et al.*, 1993; Ward and Nowell, 1997). *Cercospora zeae-maydis* was first identified and named by Tehon and Daniels (1925) in southern Illinois in the USA. More recent reports by Hilty *et al.* (1979) and Latterell and Rossi (1983) have shown that this fungus can cause yield losses of up to 27.7%. Yield losses are correlated with the length of time that leaf-blighting is present during grain-fill: the longer the period of blighting before physiological maturity, the greater the yield losses. Hilty *et al.* (1979), Latterell and Rossi (1983), Ayres *et al.* (1985), Smith (1989) and Jenco (1995) showed that this fungus can cause grain yield losses of up to 79%. However, grain yield losses are usually from 0 - 30% (Hilty *et al.*; 1979; Latterell and Rossi, 1983; Ayres *et al.*, 1985; Stromberg and Donahue, 1986; Donahue *et al.*, 1991; Lipps and Pratt, 1991; Martinson *et al.*, 1994; Wegulo, 1994; Nutter *et al.*, 1995; Jenco, 1995). Stromberg and Donahue (1986), using a disease index of 1 - 5, showed that for each one unit change in the disease index, longer maturing hybrids lost 1.06 t ha^{-1} and lodging increased by 12.4%, average maturing season hybrids lost 0.7 t ha^{-1} and lodging increased by 4.0%, and early maturing hybrids decreased in yield by 0.1 t ha^{-1} and lodging increased by 7.5%. Shurtleff and Pedersen (1991) reported losses of up to 100% due to stalk deterioration (could not be mechanically harvested) following severe GLS infection in 1973 and 1974 in Virginia. This has been substantiated by other researchers (Roane *et al.*, 1974; Latterell and Rossi, 1983; Smith, 1989).

In seed crops in Iowa, USA, it was found that the total grain yield and the saleable seed fraction were significantly reduced by *C. zeae-maydis* infection. Seed losses of between 7.7% and 25.1% occurred in two trials in Iowa. The application of fungicides increased all categories of seed size, although the increases were greatest in the smaller seed size categories, and the total grain yield increased significantly (Rivera-Canales, 1993).

The economic losses in grain yield due to GLS have not been well quantified in South Africa, although preliminary trials by Ward *et al.* (1993) and Ward and Nowell (1997) suggested losses ranged between 0 and 60%. The research described below was undertaken to more accurately determine the scale of losses sustained under South African conditions.

8.2 Materials and Methods

The trials were conducted at Pannar Seed (Pty.) Ltd near Greytown (29°02'S, 30°31'E and an altitude of 1100 m), and at the Cedara Agricultural Development Institute at Cedara, (29°31'S, 30°17'E and an altitude of 1070 m), in KwaZulu-Natal, South Africa.

8.2.1 Fungicide trials

The trial at Cedara had conducted been on a sandy clay loam of the Hutton form and Doveton series soil (MacVicar, 1991) which was previously under monoculture no-till maize. A GLS-susceptible hybrid, RS 5206, was direct-drilled on 26 November 1992 into land that had been chisel ploughed twice (surface stubble was assessed to be >60%, using the sighting frame described by Lang and Mallet [1982]). Fertilizer for an 8 t ha^{-1} grain crop was applied according to the soil analysis. A top dressing of 98 kg N ha^{-1} was applied when the maize was knee high. A pre-plant application of 2.0 ℓ ha^{-1} of Sting SL (glyphosate, 180 g ℓ^{-1}) and 200 ℓ ha^{-1} of Decis EC (deltamethrin, 25 g ℓ^{-1}), and a post emergence treatment of 2.0 ℓ ha^{-1} of Dual 930S EC (metolachlor, 930 g ℓ^{-1}) were applied to control cutworm and weeds. Each plot consisted of eight 9m rows, spaced 750mm apart. The trial was planted as a randomised blocks design of 10 treatments (the common fungicides at Cedara and Greytown were difenoconazole [87g ha^{-1}], flutriafol [156.25g ha^{-1}], carbendazim/flusilazole [187.5g/93.8g ha^{-1}], carbendazim/flutriafol [187.5g/117.5g ha^{-1}] and benomyl [375g ha^{-1}]), replicated three times.

The first experiment was planted on 26/11/92 and the central four rows of each plot were sprayed three times every 21 days with the fungicides, with the first fungicide application being seven days before tassel emergence and 14 days after the first GLS lesions were observed. The fungicides common to Cedara and Greytown were difenoconazole (87.5g a.i. ha^{-1}), flutriafol (156.25g a.i. ha^{-1}), carbendazim / flusilazole (187.5g/93.8g a.i. ha^{-1}), carbendazim / flutriafol (187.5g/117.5g a.i. ha^{-1}) and benomyl (375g a.i. ha^{-1}). The second fungicide experiment was planted on 17 November 1992 and the central four rows were sprayed four times every 21 days, with the first application being ten days before pollen shed when GLS lesions were observed on the lower four to five leaves. In this experiment, the fungicides common to Cedara and Greytown were difenoconazole (87.5g a.i. ha^{-1}), flutriafol (156.25g a.i. ha^{-1}), carbendazim / flusilazole (187.5g/93.8g a.i. ha^{-1}), carbendazim / flutriafol (187.5g/117.5g a.i. ha^{-1}) and benomyl (375g a.i. ha^{-1}). Fungicides were applied in 450 ℓ ha^{-1} of water. Leaf assessments

were done as a percentage leaf area loss due to GLS. The trial was hand harvested on 11 June 1993 and the yield was adjusted to 12.5% grain moisture.

In Greytown, the trial was conducted on a Hutton soil which had previously been ploughed and monocropped to maize. The GLS-susceptible hybrids, PAN 6528 and PNF 6552, were hand-planted on 20 November 1992 and 23 November 1993, respectively. Fertilizer sufficient for an 8 t ha^{-1} grain crop was applied. A top dressing of 89 kg N ha^{-1} was applied when the maize was knee high. A pre-plant herbicide of 4 ℓ ha^{-1} of Eptam Super (EPTC, 720 g ℓ^{-1}), a pre-emergence insecticide of 140 mℓ ha^{-1} of Tralate (tralomethrin, 36 g ℓ^{-1}), and a post emergence herbicide of 1.2 ℓ ha^{-1} of Galleon (atrazine and sulcotrione, 300 g $\ell^{-1}$and 125 g ℓ^{-1}; respectively) were applied to control cutworm and weeds. The trial design was a randomised complete blocks design of 20 treatments (6 fungicides were common to Cedara and Greytown over seasons), replicated three times and each plot consisted of two rows of 4.4 m, spaced 910 mm apart. Spacing between plants in the row was 22cm. Each plot was separated with a single border row to reduce fungicide drift between plots. The experiments were first sprayed on 10 December 1992 and 10 January 1993, respectively. The common fungicides used in the trial at Greytown were flutriafol (125g a.i. ha^{-1}), carbendazim / flusilazole (250g/125g a.i. ha^{-1}), benomyl (250g a.i. ha^{-1}), difenoconazole (75g a.i. ha^{-1}) and carbendazim / flutriafol (150g/90g a.i. ha^{-1}). The first fungicide application was seven days after the first GLS lesions were observed, which was approximately a week before tassel emergence. Fungicides were applied three times at 14 day intervals in 400 ℓ ha^{-1} of water by Matabi® knapsack sprayer at 2 bars pressure.

Whole-plant standard area diagrams described by Ward *et al.* (1996) were used as a guide in estimating disease severity. The trial was hand-harvested on 16 May 1994 and 08 June 1994, and the yields adjusted to 12.5% grain moisture. Grain yield loss was determined by comparing the non-sprayed control to the most effective fungicide (representing the expected yield without GLS present).

8.2.2 Hybrid trials

Maize hybrids submitted to the South African National Maize Cultivar Phase II Trial series were evaluated for their susceptibility to GLS disease during the 1991/92, 1992/93 and 1993/94 growing seasons. The experiments comprised 49 hybrids each season, laid out in a 7 x 7 triple

lattice design of 3 replicates. The trials at Cedara were repeated under both conventional- and stubble-tillage systems. At Greytown, the trial site was conventionally tilled only. The conventional-tillage treatment was disced in the winter, mouldboard ploughed in September and finally disced immediately before planting to incorporate the previous season's crop residue. The stubble treatment was chisel-ploughed to a depth of 120mm in the winter and again prior to planting. Chisel-plough tines were spaced 310mm apart and fitted with sweeps. After tillage the residue cover on stubble treatments was 31%. Planting lines were drawn immediately prior to planting when fertilizer sufficient for an 8 t ha^{-1} grain crop was band applied. A topdressing of 100 kg N ha^{-1} was broadcast when maize was between the eight- and ten-leaf stage. Normal weed and pest control practices (as described in section 8.2.1) were followed as for the fungicide trials. 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 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}. The harvested area of two, 6.0 m rows, was hand-harvested. At Greytown, plots were two rows 4.4 m long and 0.9 m apart, were hand-planted in early October, and hand-thinned to 50 000 plants ha^{-1}. The whole plots were hand-harvested.

Whole-plant standard area diagrams described by Ward *et al.* (1997a) were used as a guide in estimating disease severity as a percentage leaf area infected. The single point model (% disease severity near physiological maturity) was used as the disease index in the linear regression analysis. Grain yields were determined and were expressed in kg ha^{-1} at 12.5% moisture. The percentage lodged plants were also determined.

8.2.3 Economic analysis

Ninety five percentage confidence limits were calculated for the loss in grain yield in order to establish upper and lower confidence limits for grain yield loss due to GLS, for both the fungicide trials and individual hybrids. The economic loss, and the upper and lower economic loss limits were determined by multiplying the yield loss / limits by the estimated average maize price of R650 t^{-1} (Saville, 1983).

8.2.4 Statistical analysis

The percentage GLS, percentage stem lodging and grain yield data from the fungicide trials were analysed by analysis of variance (ANOVA). Fischer's least significant differences were based on the 5% level of probability. The analysis was conducted using Genstat 5.31.

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

$$Y = B_o + B_i X_i + E_i$$

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), X_i 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 locations, seasons, tillage practices GLS resistance groupings and selected individual hybrids. The regression analysis was conducted on Genstat 5.31 and Statsgraphics 4.0. Bartlett's χ^2 test was used to test for homogeneity of variance.

8.3 Results

The growing conditions in 1991/92 were excellent during the vegetative growth stages of the maize, but rainfall declined after anthesis and it was dry during grain-fill. However, heavy dews were frequent during this period, which ensured disease development. The 1992/93 season was dry, with only 50% of the mean rainfall 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 January and February.

8.3.1 Fungicide trials

Results are presented in Tables 8.1 - 8.4. From these data it can be seen that there was significant variation in efficacy between fungicides in controlling GLS, although this was seldom significant when grain yield was determined. However, the overall trend between sites and over seasons was similar.

Table 8.1: Grain yield (t ha^{-1}), grain yield loss (t ha^{-1}), economic loss, percentage stem lodging, and the percentage leaf area loss due to GLS for the fungicide trial in 1992/93 at Cedara

Active Ingredient	Rate Active ha^{-1}	Yield (Y) t ha^{-1}	% Yield Loss	% Stem Lodging	% GLS[a]	Yield loss over best treatment t ha^{-1}			Economic loss [d] R ha^{-1}		
						Actual	Lower limit [b]	Upper limit [c]	Actual	Lower limit	Upper limit
Difenoconazole	87.5g	7.731 b	0.0	6.0	32.5 c	0			0		
Flutriafol	156.25g	7.723 b	1.0	5.7	12.8 def	0.008	0.909	-0.893	5.20	590.85	-580.45
Carbendazim / flusilazole	187.5g/93.8g	7.723 b	1.0	6.8	4.2 f	0.008	0.909	-0.893	5.20	590.85	-580.45
Carbendazim / flutriafol	187.5g/117.5g	7.568 b	2.1	8.3	7.6 ef	0.163	1.064	-0.738	105.92	691.60	-479.70
Benomyl	375g	7.319 b	5.3	6.1	18.1 de	0.412	1.313	-0.489	267.80	853.45	-317.85
Non-sprayed control		4.227 a	45.3	5.6	83.7 a	3.504	4.405	2.603	2277.60	2863.25	1691.95
Mean		7.062		6.7	25.6						
%C.V.		8.8		8.8	32.4						
LSD $_{0.05}$		0.901		N.S.	12.1						

[a] Assessment undertaken 131 days after planting as a % leaf area loss.
[b] Lower-gain was calculated from the 95% confidence limit.
[c] Upper-gain was calculated from the 95% confidence limit.
[d] Economic loss equals the loss in grain yield multiplied by the maize price of R650 $tonne^{-1}$.

Table 8.2: Grain yield (t ha^{-1}), grain yield loss (t ha^{-1}), economic loss, percentage lodging, and the percentage leaf area loss due to GLS for the fungicide trial in 1993/94 at Cedara

Active Ingredient	Rate Active ha^{-1}	Yield (Y) t ha^{-1}	% Yield Loss	% Stem Lodging	% GLS[a]	Yield loss over best treatment t ha^{-1}			Economic loss [d] R ha^{-1}		
						Actual	Lower limit [b]	Upper limit [c]	Actual	Lower limit	Upper limit
Carbendazim / flutriafol	187.5g/117.5g	7.810 b	0.0	6.9 b	33.1 c	0			0		
Carbendazim / flusilazole	187.5g/93.8g	7.411 b	5.1	7.3 ab	26.3 c	0.399	1.352	-0.554	259.35	878.80	-360.10
Difenoconazole	87.5g	7.205 b	7.8	8.4 ab	53.8 b	0.605	1.558	-0.348	393.25	1012.70	-226.20
Flutriafol	156.25g	6.994 b	10.4	7.0 b	34.4 c	0.816	1.769	-0.137	530.40	1149.85	-89.05
Benomyl	375g	6.964 b	10.8	10.1 ab	28.1 c	0.846	1.799	-0.107	549.90	1169.35	-69.55
Non-sprayed control		4.858 a	37.8	15.2 a	90.0 a	2.952	3.905	1.999	1918.80	2538.25	1299.35
Mean		6.779		10.5	44.3						
%C.V.		9.7		58.4	14.6						
LSD $_{0.05}$		0.953		7.9	9.4						

[a] Assessment undertaken 131 days after planting as a % leaf area loss.
[b] Lower-gain was calculated from the 95% confidence limit.
[c] Upper-gain was calculated from the 95% confidence limit.
[d] Economic loss equals the loss in grain yield multiplied by the maize price of R650 $tonne^{-1}$.

Table 8.3: Grain yield (t ha^{-1}), grain yield loss (t ha^{-1}), economic loss, percentage lodging, and the percentage leaf area loss due to GLS for the early fungicide trial in 1992/93 at Greytown

Active Ingredient	Rate Active ha^{-1}	Yield (Y) t ha^{-1}	% Yield Loss	% Stem Lodging	% GLS[a]	Yield loss over best treatment t ha^{-1}			Economic loss [d] R ha^{-1}		
						Actual	Lower limit [b]	Upper limit [c]	Actual	Lower limit	Upper limit
Flutriafol	125g	7.94 d	0.0	28.0	1.5 b	0			0		
Carbendazim / flusilazole	250g/125g	7.30 bcd	8.1	21.2	0.8 b	0.64	1.84	-0.56	416.00	1196.00	-364.00
Benomyl	250g	6.87 bcd	13.5	23.5	3.0 b	1.07	2.27	-0.13	695.50	1475.50	-84.50
Difenoconazole	75g	6.48 abc	18.4	14.3	2.5 b	1.46	2.66	0.26	949.00	1729.00	169.00
Carbendazim / flutriafol	150g/90g	6.40 ab	19.4	21.9	1.2 b	1.54	2.74	0.34	1001.00	1781.00	221.00
Non-sprayed control		5.55 a	30.1	28.0	41.2 a	2.39	3.59	1.19	1553.50	2333.50	773.50
Mean		6.13		28.4	10.8						
%C.V.		13.9		58.2	40.9						
LSD $_{0.05}$		1.20		N.S.	6.3						

[a] Assessment undertaken 131 days after planting as a % leaf area loss.
[b] Lower-gain was calculated from the 95% confidence limit.
[c] Upper-gain was calculated from the 95% confidence limit.
[d] Economic loss equals the loss in grain yield multiplied by the maize price of R650 $tonne^{-1}$.

Table 8.4: Grain yield (t ha^{-1}), grain yield loss (t ha^{-1}), economic loss, percentage lodging, and the percentage leaf area loss due to GLS for the late fungicide trial in 1993/94 at Greytown

Active Ingredient	Rate Active ha^{-1}	Yield (Y) t ha^{-1}	% Yield Loss	% Lodging	% GLS[a]	Yield loss over best treatment t ha^{-1}			Economic loss[d] R ha^{-1}		
						Actual	Lower limit[b]	Upper limit[c]	Actual	Lower limit	Upper limit
Flutriafol	250g	5.540 b	0.0	46.8 c	8.5 bc	0			0		
Carbendazim / flusilazole	150g/90g	5.225 b	5.1	48.0 c	6.8 bc	0.315	1.100	-0.470	204.75	715.00	-305.50
Carbendazim / flutriafol	250g/125g	5.080 b	8.3	50.7 bc	7.2 bc	0.460	1.245	-0.325	299.00	809.25	-211.25
Difenoconazole	75g	5.005 b	9.7	69.3 bc	13.0 b	0.535	1.320	-0.250	347.75	858.00	-162.50
Benomyl	125g	4.630 b	16.4	72.5 b	2.8 c	0.910	1.695	0.125	591.50	1101.75	81.25
Non-sprayed control		2.885 a	47.9	98.6 a	38.2 a	2.655	3.440	1.870	1725.75	2236.00	1215.50
Mean		4.085		74.8	19.6						
%C.V.		13.6		22.2	23.2						
LSD $_{0.05}$		0.785		23.4	6.4						

[a] Assessment undertaken 131 days after planting as a % leaf area loss.
[b] Lower-gain was calculated from the 95% confidence limit.
[c] Upper-gain was calculated from the 95% confidence limit.
[d] Economic loss equals the loss in grain yield multiplied by the maize price of R650 $tonne^{-1}$.

All fungicides, in both seasons, significantly reduced the severity of GLS. Table 8.1 shows that the grain yield loss at Cedara was 45.3% (3.504 t ha^{-1}) associated with a GLS severity of 83.7% for 1992/93. This is equivalent to a loss of R2 277.60 ha^{-1}, with a lower limit of R1 691.95 ha^{-1} and an upper limit of R2 863.25 ha^{-1}. The GLS severity of the non-sprayed control was 150% higher than the worst fungicide treatment (difenoconazole). However, it is of interest to note that although GLS severity ranged from 4 - 33% for the various fungicide treatments, there were no significant differences in grain yield between treatments. There were no significant differences in stem lodging between treatments.

Even with the use of fungicides, the severity of GLS was high in 1993/94 at Cedara. Table 8.2 shows that the grain yield loss at Cedara was 37.8% (2.952 t ha^{-1}) associated with a GLS severity of 90% for 1993/94. This is equivalent to a loss of R1 918.80 t ha^{-1}, with a lower limit of R1 299.35 and an upper limit of R2 538.25 ha^{-1}. The GLS severity of the non-sprayed control was about 90% higher than the least effective fungicide treatment. Grey leaf spot severity ranged from 26 - 54% but there were no significant differences in grain yield between treatments.

Difenoconazole and flutriafol significantly reduced stem lodging when compared to the non-sprayed control despite a high coefficient of variation. These treatments had over 50% fewer lodged plants than the non-sprayed control.

Table 8.3 showed that grain yield loss at Greytown was 30.1% (2.390 t ha^{-1}) associated with a GLS severity of 41.2% in 1992/93. This is equivalent to a loss of R1 533.50 ha^{-1}, with a lower limit of R771.50 and a higher limit of R2 333.50 ha^{-1}. The non-sprayed control was not lower-yielding than the carbendazim/flutriafol and difenoconazole treatments. Benomyl, carbendazim/flusilazole and flutriafol had higher grain yields than the non-sprayed control. The highest yield was obtained by the flutriafol treatment. In this case these differences in grain yield occurred with GLS severity levels of 0.8 - 3.0%.

No differences in lodging were found between fungicide treatments and the non-sprayed control in 1992/93.

The yield potential of PNF 6552, which was planted in 1993/94 at Greytown, was lower than of PAN 6528, which was planted in 1992/93. Grain yield loss at Greytown was 47.9% (2.655

t ha^{-1} in total) with an associated GLS severity of 38.2% in 1993/94 (Table 8.4). This is equivalent to a loss of R1 725.75 ha^{-1}, with a lower limit of R1 215.50 and an upper limit of R2 236.00 ha^{-1}. There were no differences in grain yield between the different fungicide treatments but they ranged from 2.8 - 13.0% in GLS severity.

All fungicide treatments resulted in fewer lodged plants than the non-sprayed control in the 1993/94 season. The most effective fungicide treatments, flutriafol and carbendazim / flusilazole, reduced the percentage lodged plants by more than 50%.

8.3.2 Regression

By regressing the percentage GLS severity against grain yield for hybrids, seasons, location and tillage systems, 66.9% of the variance was accounted for. The mean grain yield loss was 55.85 $\pm$2.27 kg ha^{-1} for each percentage increase in GLS severity. Results are presented in Tables 8.5 - 8.8.

The effect of tillage was determined only at Cedara, as Greytown was conventionally-tilled. The 1992/93 season showed significant heterogeneity of variance (χ^2=47.9, $P \leq 0.01$) for GLS severity. For this reason 1992/93 data was not used in determining yield loss due to GLS. Stubble-tillage resulted in a significantly higher grain yield in 1991/92 and 1992/93, but with well distributed rainfall in 1993/94, there were no differences in grain yield between tillage treatments (Table 8.5). Under stubble-tillage (Table 8.6) there was a decrease of 32.3 kg ha^{-1} in grain yield for each percentage increase in disease severity (a total grain loss of 1.778 t ha^{-1}). Under conventional-tillage there was a higher yield decrease of 34.8 kg ha^{-1} for each percentage increase in leaf blighting (a total grain loss of 1.734 t ha^{-1}). The lower yield decrease under stubble-tillage was in contrast to a higher mean GLS severity of 55.0%, compared to 49.8% GLS under conventional tillage (Table 8.5). Under conventional tillage, grain yields over both locations were 1266 kg ha^{-1} higher in the 1991/92 than the 1993/94 season. The difference was highly significant ($P \leq 0.001$). Yield loss in 1991/92 was 11.0 kg ha^{-1} for each percentage increase in GLS severity in contrast with 40.2 kg ha^{-1} during the 1993/94 season (Table 8.6).

Table 8.5: Effect of conventional and stubble-tillage treatments on grey leaf spot severity and grain yield at Cedara and Greytown over 1991/92 and 1993/94 seasons (Ward *et al.*, 1997c)

SOURCE	EFFECT	SEASON		
		1991/92	1993/94	MEAN
Disease Severity (%)[1]				
Tillage	Conventional	32.08	67.57	49.83
	Stubble	26.42	83.58	55.05
	F-test	N.S.[2]	N.S.	N.S.
	Standard Error	1.55	7.44	3.80
	% C.V.	37.1	15.4	21.5
Location	Cedara	32.08	67.57	49.83
	Greytown	14.69	59.01	36.85
	F-probability	*	N.S.	*
	Standard Error	3.50	8.90	3.80
	% C.V.	33.4	14.9	11.9
Grain Yield (t ha^{-1})				
Tillage	Conventional	7.557	4.798	6.177
	Stubble	8.161	4.480	6.321
	F-test	*	N.S.	N.S.
	Standard Error	0.078	0.158	0.326
	% C.V.	6.8	11.6	8.6
Location	Cedara	7.557	4.798	6.177
	Greytown	9.389	6.922	8.156
	F-test	**	**	**
	Standard Error	0.096	0.085	0.064
	% C.V.	8.0	8.9	8.4

1 Disease severity is the percentage leaf blighting assessed approximately 25 days before physiological maturity.

2 N.S. = non-significant., * = 5% level of significance, and ** = 1% level of significance.

Table 8.6: Predicted yield and yield loss of all hybrids with grey leaf spot during the 1991/92 and 1993/94 seasons

Category	Yield potential (t ha^{-1})	Yield loss [a] (kg ha^{-1})	R^2
Tillage (Cedara only)			
Conventional tillage	6177	-34.8	84
Stubble tillage	6321	-32.3	88
Location			
Greytown	9721	-30.4	65
Cedara	7632	-37.1	83
Season			
1991/92	7813	-11.0	71
1993/94	6547	-40.2	80

[a] Yield loss in kg ha^{-1} for each percentage increase in GLS severity.

Over locations, the variance of GLS severity was heterogeneous (χ^2=65.45, P≤0.01) and a weighted analysis showed that GLS severity was consistently higher at Cedara (49.8%) than at Greytown (36.8%). Variance in grain yield was homogeneous (χ^2=4.94, N.S.), which indicated that the effect of GLS on grain yield was similar at both locations. Grain yield at Greytown (8.516 t ha^{-1}) was consistently higher than at Cedara (6.177 t ha^{-1}). This is not surprising, in view of the lower disease levels at Greytown. At Cedara, there was a larger yield decrease of 37.1 kg ha^{-1} for each percentage increase in leaf blighting, whilst at Greytown the yield decrease was 30.4 kg ha^{-1} for each percentage increase in leaf blighting (Table 8.6).

Table 8.7: Predicted yield and yield loss for two grey leaf spot susceptibility categories at Greytown and Cedara in 1991/92 and 1993/94 under conventional tillage only

Category	Yield potential t ha-1	Yield loss [c] kg ha^{-1}	R^2	%GLS [d]	Total Loss [e] t ha^{-1}	% Yield Loss
Over locations						
Susceptible [a]	10.272	-66.2	90	58	3.840	37
More resistant [b]	10.188	-19.9	88	26	0.517	5
Overall	7.935	-34.8	84	37	1.288	16
Greytown only						
Susceptible [a]	10.571	-57.4	78	51	2.927	28
More resistant [b]	10.144	-16.7	66	23	0.384	4
Overall	9.721	-30.4	65	39	1.186	12
Cedara only						
Susceptible [a]	9.876	-68.9	87	64	4.410	45
More resistant [b]	9.643	-26.7	74	34	0.908	9
Overall	7.632	-37.1	83	50	1.855	24

[a] Susceptible - The hybrids CRN 4523, PAN 6364, PAN 6528, PAN 6552, RO 430, RS 5206, SC 5232, SC 5240, SNK 2340, SNK 2888 and SNK 2950 which were assessed as being most susceptible to GLS (based on leaf area loss over the whole plant).

[b] More resistant - The hybrids CRN 3584, NS 9100, PAN 6363, PAN 6364, PAN 6479, PAN 6480, PAN 6549, PAN 6578, SNK 2147 and SNK 2665, which were assessed as being most resistant to GLS (based on leaf area loss over the whole plant).

[c] Yield loss for each percentage increase in GLS severity.

[d] Mean percentage GLS infection, based on whole plant assessments, for the 1991/92 and 1993/94 seasons.

[e] Total grain yield losses in t ha^{-1} and percentage loss in brackets.

Table 8.8: Regression coefficients, for individual hybrids, for grain yield against grey leaf spot for a range of hybrids, ranked according to percentage grain yield loss, at Cedara and Greytown in 1991/92 and 1993/94, and the estimated economic losses (individual hybrid data from Table 8.7)

						Predicted yield loss in tonnes ha^{-1}			Predicted economic loss [f] in Rand ha^{-1}		
Hybrid	Yield potential [a] t ha^{-1}	Yield loss [b] kg ha^{-1}	R^2	%GLS [c]	% Yield loss	Actual	Lower limit [d]	Upper limit [e]	Actual	Lower limit	Upper limit
PAN 6480	10.081	-53.6	52	24	13	1.286	0.730	1.841	835.90	474.50	1196.65
PAN 6479	9.693	-81.1	69	17	14	1.379	0.958	1.799	896.35	623.00	1169.09
CRN 3584	10.123	-56.3	79	28	16	1.576	0.995	1.998	1024.40	646.65	1298.57
N 9100	8.825	-47.3	48	30	16	1.419	0.751	2.089	922.35	488.28	1357.98
PAN 6549	9.413	-56.6	81	32	19	1.811	1.410	2.209	1177.15	916.24	1436.03
PAN 6578	9.594	-62.6	69	30	20	1.878	1.294	2.459	1220.70	841.23	1598.61
PAN 6364	9.564	-49.7	74	40	21	1.988	1.435	2.538	1292.20	932.62	1649.44
SNK 2888	10.006	-50.3	77	50	25	2.515	1.885	3.143	1634.75	1225.25	2042.95
A 1849	9.993	-67.6	65	46	31	3.110	2.054	4.163	2021.50	1335.33	2706.25
SNK 2950	9.131	-53.4	66	54	32	2.884	2.313	3.765	1874.60	1503.68	2447.52
CRN 4605	9.370	-56.4	77	57	34	3.215	2.397	4.026	2089.75	1558.33	2617.21
PAN 6528	9.628	-64.8	83	52	35	3.370	2.669	4.075	2190.50	1734.62	2648.91
RS 5206	11.033	-73.6	82	53	35	3.901	3.055	4.742	2535.65	1986.04	3082.24
PAN 6552	10.960	-66.1	70	61	37	4.032	2.818	5.248	2620.80	1831.43	3411.09

[a] Potential yield in tonnes ha^{-1}.
[b] Predicted yield loss.
[c] Assessment undertaken 131 days after planting as a % leaf area loss.
[d] Lower-gain was calculated from the 95% confidence limit.
[e] Upper-gain was calculated from the 95% confidence limit.
[f] Economic loss equals the loss in grain yield multiplied by the maize price of R650 tonne^{-1}.

Hybrids were grouped into either susceptible or more resistant categories (using Gupta's Bestest [Gupta, 1965; Calitz, 1991; van Aarde, 1993 and 1994]) based on GLS severity over the two seasons. Large differences in grain yield loss between the two groups were obvious (Table 8.7). Overall, the susceptible group lost 66.2 kg ha^{-1} for each percentage increase in GLS severity (3.840 t ha^{-1} in total) and the more resistant group lost only 19.9 kg ha^{-1} for each percentage increase in GLS severity (0.517 t ha^{-1} in total). Grain yield losses at Greytown are consistently smaller than those at Cedara but the grain yield trends were similar (Table 8.7).

There were differences in individual hybrid responses to GLS (Table 8.8). The more GLS-susceptible hybrids, PAN 6552 and RS 5206, lost 66.1 kg ha^{-1} and 73.6 kg ha^{-1} for each percentage increase in GLS severity or 4.032 t ha^{-1} and 3.901 t ha^{-1} in total, respectively. PAN 6364 and SNK 2950 only lost 49.7 kg ha^{-1} and 53.4 kg ha^{-1} for each percentage increase in GLS severity or 1.988 t ha^{-1} and 2.884 t ha^{-1} in total, respectively. PAN 6479, with the least GLS, had a yield loss of 81.1 kg ha^{-1} (1.379 t ha^{-1} in total) for each percentage increase in GLS severity. The more GLS-resistant hybrids such as PAN 6480 and CRN 3584 lost 53.6 kg ha^{-1} and 56.3 kg ha^{-1} for each percentage increase in GLS severity or 1.286 t ha^{-1} and 1.576 t ha^{-1} in total, respectively. NS 9100 lost 47.3 kg ha^{-1} for each percentage increase in GLS severity (1.419 t ha^{-1} in total) but only 48% of the variance in yield was accounted for.

When converted into economic terms, the least GLS-susceptible hybrid, PAN 6480, showed a loss of R835.90 ha^{-1} (with a range of R474.50 - R 1 196.65 ha^{-1}). The most GLS-susceptible hybrid PAN 6552, showed a loss of R2 620.80 ha^{-1} (with a lower limit of R1 831.43 and an upper limit of R3 411.09 ha^{-1}). The losses of the other hybrids were dispersed within this range.

8.4 Discussion

The fungicide trials showed grain yield losses to be slightly less than those initially reported by Ward *et al.* (1993), Ward and Nowell (1997) and Ward *et al.* (1997a and 1997b). Losses over the two seasons, including the drought season in 1992/93, ranged from 30 - 48% of the potential yield (as determined by the application of fungicides) at both sites (see Table 8.9).

Table 8.9: Summary of fungicide yield loss data, based on Tables 8.1 - 8.4

Location	Season	GLS (%)			Yield Loss (%)
		Fungicides	Control	Difference	
Cedara	1992/93	4 - 33	84	51	45
	1993/94	26 - 54	90	36	38
Greytown	1992/93	1 - 3	41	38	30
	1993/94	3 - 13	38	25	48

Table 8.10:- A summary of the economic losses as determined from the grain yield of non-sprayed control plots for the 1992/93 and 1993/94 seasons at Greytown and Cedara, based on Tables 8.1 - 8.4

Season	Economic loss (R ha^{-1})		
	Greytown	Cedara	Mean
1992/93	1554	2278	1916
1993/94	1726	1919	1822
Mean	1640	2098	1869

Table 8.10 summarises the estimated economic losses, based on the fungicide trials, over locations and seasons. These losses were determined using the average maize price obtained by the farmer in KwaZulu-Natal for the 1996. It is assumed that the maize grain price will be similar in 1997. The economic value of the grain yield losses ranged from R1 554 to R2 278 ha^{-1} over locations and seasons. The average economic loss at Greytown was R1 640 ha^{-1} and at Cedara was R2 098 ha^{-1}. It was surprising to note that the mean grain yield loss during 1992/93 was 90 kg ha^{-1} (R58 ha^{-1}) more than during the 1993/94 season. This was largely due to the lower yield loss at Cedara than at Greytown during the 1993/94 season. This is likely to be the result of the fungicide treatments not completely controlling GLS (Table 8.2 and 8.4). The mean loss, over both locations and seasons, to the farmer was R1 869 ha^{-1} (2.875 t ha^{-1}). On farms growing 100 ha of maize (many commercial farmers in KwaZulu-Natal produce in excess of this) this translates into a loss of R186 900 per annum. Such losses could be financially disastrous.

The cost of each fungicide application is R195.09 ha^{-1} or equivalent to 300 kg ha^{-1} of grain yield

at the current producers price of maize at R650 ha^{-1} (Table 8.11). Should a farmer have to apply fungicide three times during the season, the cost would be R585.27 ha^{-1} or 900 kg ha^{-1} of grain. Based on the estimated value of grain losses due to GLS in Table 8.9, controlling GLS on susceptible hybrids with fungicides is of economic benefit to the maize farmer. The risks and timing of fungicide application are dealt with in detail by Ward *et al.* (1997b).

Table 8.11: Application costs of registered fungicides to maize in the Winterton and Karkloof regions of KwaZulu-Natal in 1995/96

Fungicide	Harvesting [a] (R t^{-1})	Fungicide (R ha^{-1})	Application [b] (R ha^{-1})	Total
Carbendazim/difenoconazole	28.73	128.18	60.50	217.41
Carbendazim/flusilazole	28.73	97.47	60.50	186.70
Carbendazim/flutriafol	28.73	91.92	60.50	181.15
Mean	28.73	105.86	60.50	195.09

[a] Harvesting costs include labour and mechanical costs of R26.12 for 1995/96 plus an estimate of 10% inflation for 1995/96.

[b] Application costs includes labour and mechanical costs of R55.00 for 1995/96 plus an estimate of 10% inflation for 1995/96.

Grain yield losses do vary between locations and seasons, but such variation is not as large as expected (the range is from 30.1 - 47.9%). A further factor to note is that not all fungicides were consistently effective in controlling GLS over locations and seasons, although grain yield was always significantly better than the non-sprayed control. However, this is likely as a result of the differences in concentration of active ingredients used at the two locations. Carbendazim/flutriafol, difenoconazole, flutriafol and benomyl were applied at higher rates of active ingredients at Cedara than at Greytown. These fungicides were more effective at Cedara than at Greytown. Benomyl and difenoconazole were less effective with a higher inoculum pressure at Cedara during 1993/94 than in 1992/93. Difenoconazole and carbendazim/flutriafol were more effective in controlling GLS under a higher inoculum pressure during 1993/94 than during 1992/93 at Greytown. Carbendazim/flusilazole was applied at a lower rate of active ingredients at Cedara than at Greytown and was the most consistently effective fungicide (regardless of concentration) with a grain yield loss range between 1.0 - 8.1%.

Grain yield losses for individual hybrids, as determined by regressions analysis, ranged from 13% - 37% (Table 8.7). However, when the hybrids were grouped into either susceptible or

more resistant groups overall, the susceptible group had a mean grain yield loss of 37% and the more resistant hybrids a mean grain yield loss of 5%. Although there are differences in yield loss between the two methods of determining yield losses through regression analysis, the results are not dissimilar. Differences in grain yield losses existed between Greytown with 12% overall (28% and 4% yield loss for the susceptible and more resistant groups, respectively) and Cedara with 24% overall (45% and 9% for the susceptible and more resistant groups, respectively). Such large differences were not apparent in the fungicide trials. It is possible that other diseases, such as *Puccinia sorghi* Schw., *Exserohilum turcicum* (Pass.) Leonard & Suggs and a *Phaeosphaeria* sp., that were more prevalent and severe at Greytown than at Cedara, could have had a confounding effect. However, at the time it was thought that the incidence and severity of these diseases did not warrant separate assessment.

When each hybrid's grain yield loss was determined, the losses were very similar in ranking to that of the percentage leaf area lost due to GLS. If the individual percentage yield losses for each percentage increase in GLS are examined in isolation, some of the highest yield losses appear to be from the more resistant hybrids, e.g. PAN 6479. However, when this factor is used in conjunction with the total leaf area lost due to GLS, grain yield loss is then the second lowest at 14%. PAN 6364, SNK 2950 and CRN 4605 showed lower yield loss per percentage increase in disease, but had a high yield loss due to the severity of GLS. This is an indication of tolerance by some hybrids to GLS, showing that it is important to look at all information before drawing conclusions about grain yield loss.

The variation in the estimate of grain yield loss for hybrids is from 1.286 t ha^{-1} (with a range of 0.730 - 1.841 t ha^{-1}) to 4.032 t ha^{-1} (with a range of 2.818 - 5.248 t ha^{-1}). This converts to R835.90 ha^{-1} (with a range of R474.50 - R1 196.65 ha^{-1}) to R 2 620.80 (with a range of R1 831.43 - R3 411.09 ha^{-1}). The range in economic losses for these hybrids indicates that:

i) there is a large amount of variation in the levels of resistance to GLS,
ii) the hybrids with the greatest level of resistance to GLS can still show economic losses,
iii) the use of fungicides to control GLS may not always be economically viable, with the inherent level of GLS resistance being a critical factor, and
iv) there is no doubt about the economic viability of controlling GLS through the use of fungicides on susceptible hybrids. Multiple applications of fungicides can also be economically justified.

The hybrids with a high level of GLS resistance should only be sprayed if the inoculum pressure is high and infection takes place near anthesis or earlier (each fungicide application costs the equivalent of a minimum grain yield increase of 300 kg ha^{-1}). Given ideal conditions for GLS development, it is unlikely that multiple applications of fungicide are economically justified.

It is not possible to provide clear GLS fungicide control guidelines to farmers due to the large number of variables involved. Based upon the above information, it can be recommended that GLS-resistant hybrids, adapted to the given region, be utilized in conjunction with other agronomic practices that reduce the severity or duration of a GLS epidemic. This includes the stringent use of fungicides. If a susceptible hybrid is planted in a high risk GLS region, then at least two fungicide applications will have to be made during the course of the season.

The decision to apply fungicides will have to be made by each farmer after considering:

i) the risk of a GLS epidemic on the farm
ii) the level of GLS resistance of the hybrid/s planted
iii) the growth stage at which GLS is first observed
iv) the amount of time left to physiological maturity
v) the prevailing weather conditions and the risk of the epidemic continuing.

Due to the large number of variables involved when applying fungicides, genetic resistance to GLS is the preferred and more simple management practice that should be followed.

It is apparent from these trials that if GLS is not adequately controlled there will be a large increase in lodging adding to the grain yield losses sustained. The fungicide trial showed that lodging increases of 100% can occur in susceptible hybrids grown without any GLS control. This would have a significant impact on the harvestable yield should mechanical harvesting be practised. Having to pick up the remaining unharvested grain by hand would impact on the profitability of the operation.

Grain yield losses in the USA were usually up to 30%, but yield losses as high as 79% have been reported (Hilty *et al.*, 1979; Latterell and Rossi, 1983; Ayres *et al.*, 1985; Stromberg and Donahue, 1986; Smith, 1989; Nutter *et al.*, 1995; Jenco, 1995). The grain yield losses found in KwaZulu-Natal, South Africa, are larger than the yield losses usually reported from the USA as a whole but were similar to those found in Virginia (Stromberg and Donahue, 1986). This shows that maize in South Africa is currently more severely affected by GLS than maize in the

USA. Yield losses in excess of those reported in these trials have been found in South Africa (Ward *et al.*, 1993; Ward and Nowell, 1997; Ward *et al.*, 1997b). This is an economically important disease in South Africa and new management practices will have to be introduced to minimise the effect GLS has on local maize production.

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CHAPTER 9

The Use of Fungicides to Control Grey Leaf Spot on Maize in South Africa [15]

ABSTRACT

Grey leaf spot (GLS) disease has caused significant annual grain yield losses in the maize industry in KwaZulu-Natal, South Africa, in the relatively short period that the disease has been present. As there are no commercial hybrids immune or highly resistant to the disease and alternative measures such as crop rotations and tillage practices have limited effects, fungicides are currently the main option available for the control of the disease. This study was to determine which fungicides and fungicide mixtures most effectively controlled GLS. Separate trials were conducted at Greytown and Cedara in the 1992/93 and 1993/94 seasons. The range of fungicides tested at Greytown was more extensive and these trials were also assessed for GLS, northern corn leaf blight (NCLB) and common rust incidence. In 1992/93, a trial was conducted at Cedara to determine the effect of different fungicides rates on the efficacy of GLS control. In both seasons and sites, most fungicides were able to reduce the incidence of GLS significantly. Protectant fungicides were generally less effective than systemic fungicides. Copper fungicides were phytotoxic to maize. Fungicides of the triazole group and mixtures of the benzimidazoles and triazole fungicides were highly effective. These provided superior disease control, longer duration of control and higher grain yield responses. The combination of these two fungicide groups, with their different modes of action, not only provides excellent control of GLS but offers the theoretical benefit of slowing down the probable development of resistance to the fungicides by the pathogen. The recommended rates of fungicides tested resulted in the optimum control of GLS and significantly higher grain yields. Lower rates resulted in significantly more disease and lower grain yields.

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[15] The data from the Cedara trials only (co-operative trials conducted by J.M.J. Ward) has been accepted for publication and will be published as: Ward, J.M.J., Laing, M.D. and Nowell, D.C. 1997. Chemical control of maize grey leaf spot. Crop Protection 16 (In Press).

9.1 INTRODUCTION

Grey leaf spot (GLS) is caused by the fungus *Cercospora zeae-maydis* Tehon and Daniels. The disease, first observed in 1988 in KwaZulu-Natal, has since spread rapidly throughout the province and to neighbouring provinces. According to Smith (1989), there can be few diseases of major crops that have become a major threat to economical crop production as rapidly as has GLS of maize (*Zea mays* L.). In 1990/91, severe economic damage to maize was reported for the first time in KwaZulu-Natal (Ward and Nowell, 1997).

Early infection of maize by *C. zeae-maydis* results in significant leaf blighting and premature death of the maize plant which significantly affects yield (Stromberg and Donahue, 1986; Nutter *et al.*, 1995). Hilty *et al.* (1979), Latterell and Rossi (1983), Ayres *et al.* (1985), Smith (1989) and Jenco (1995) reported that GLS can cause grain yield losses of up to 79%. However, grain yield losses are usually from 0 - 30% (Hilty *et al.*; 1979; Latterell and Rossi, 1983; Ayres *et al.*, 1985; Donahue *et al.*, 1991; Lipps and Pratt, 1991; Martinson *et al.*, 1994; Wegulo, 1994; Jenco, 1995). Stromberg and Donahue (1986), using a disease index of 1 - 5, showed that for each unit change in a disease index, losses can range from 0.10 - 1.06 t ha^{-1}, depending on the time to maturity of the hybrid. In addition, lodging ranged from 4.0 - 12.4%. Grain yield losses in South Africa range from 0 - 60% (Ward *et al.*, 1993; Ward and Nowell, 1997).

The only known host of *C. zeae-maydis* is maize and the pathogen overwinters in colonised maize residues (Beckman and Payne, 1982; Latterell and Rossi, 1983). Not surprisingly, the increased incidence and prevalence of GLS has been linked to monoculture maize and conservation tillage practices that leave colonised maize residues on the soil surface (Rupe *et al.*, 1982; Stromberg and Donahue, 1986; Payne *et al.*, 1987; de Nazareno *et al.*, 1993; Anderson, 1995; Perkins *et al.*, 1995). Tillage practices aimed at the complete burial of colonised maize residues have been demonstrated to be a means of controlling GLS (Payne and Waldron, 1983; Huff *et al.*, 1988). However, more recently in the United States, the disease has been observed to have moved from reduced tillage situations to become a problem in fields where traditional conventional tillage is practised (Perkins *et al.*, 1995). Rotations with non-host crops is an alternative solution to ploughing, as the pathogen does not survive beyond a year in infected debris (Latterell and Rossi, 1983; Stromberg, 1986). In South Africa, few farmers practise any form of rotational cropping with maize (Channon and Farina, 1991) and rotations are unlikely to be used as an effective means of managing GLS. Genetic resistance, a highly efficient and

cost-effective method of control, is likely to provide the long-term solution to the problem (Lipps and Pratt, 1989), but no high yielding commercial hybrids completely resistant to GLS are presently available in South Africa. Chemical control measures, therefore, offer an interim solution (Ward and Nowell, 1997). Fungicide trials in the 1991/92 season, conducted at the Cedara Agricultural Development Institute (CADI) near Pietermaritzburg, South Africa, showed that GLS is capable of reducing grain yields by 20 - 60%. Some systemic fungicides were found to provide excellent control (Ward *et al.*, 1993).

Due to the lack of effective controls measures and the significant yield losses being experienced in Natal KwaZulu-Natal, this investigation was undertaken to establish which fungicides and mixtures of fungicides were most effective for the control of GLS over seasons and locations.

9.2 Materials and methods

Greytown

The trials were conducted near Greytown (29° 02'S, 30° 37'E at an altitude of 1100m), in South Africa. The site was conventionally tilled (mouldboard ploughed and disced) and soils were well-drained, sandy-clay loams of the Hutton form and Doveton series (MacVicar, 1991). Maize had been grown in this field for the past 8 years. Planting lines were drawn immediately prior to planting when fertilizer sufficient for an eight-ton grain crop ha^{-1} was band applied. A topdressing of 90 kg N ha^{-1} was broadcast when maize was between the eight- and ten-leaf stage. Normal weed- and pest-control practices were followed for the two growing regions. The trials were jab-planted by hand on 15 October 1992 and 21 October 1993 with two seeds per planting station to PAN 6528 and PNF 6552, respectively. Both trials were planted as randomised blocks, replicated three times. Plots were two rows, 4.4 m long and 0.9 m apart and thinned by hand 30 days after planting to 50 000 plants ha^{-1}. The trials were hand harvested.

Temperature and rainfall were measured during the two seasons (Tables 9.1 and 9.2).

Table 9.1: Rainfall and temperature at Greytown for the 1992/93 and 1993/94 growing seasons

	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Total / Mean
Total Rainfall (mm)													
1992/93	0.5	14.2	14.2	39.6	52.0	52.2	114.0	97.3	174.8	33.2	38.6	0.0	630.6
1993/94	0	25.3	30.3	123.6	63.3	144.6	115.0	75.6	110.1	40.9	3.9	5.9	738.5
Long Term[1] Total	10.0	28.6	54.9	85.7	77.5	121.8	124.1	99.6	97.6	26.4	20.9	14.3	761.4
Mean Temperature (°C)													
1992/93 Maximum	21.1	19.7	23.4	24.7	25.3	27.7	27.7	25.2	25.4	24.4	22.3	19.6	23.9
Minimum	3.1	5.3	10.9	12.5	13.5	16.2	15.6	15.2	13.6	11.3	7.7	3.2	10.7
1993/94 Maximum	21.2	21.0	24.5	23.0	24.5	25.7	25.7	25.2	25.5	23.7	22.7	19.6	23.5
Minimum	5.4	6.3	10.3	12.9	12.9	15.0	15.6	14.8	14.2	11.4	6.3	3.7	10.7
Long Term[1] Mean (°C)													
Maximum	18.0	19.5	21.1	21.2	22.5	23.8	24.2	24.1	23.2	22.1	20.2	17.4	21.4
Minimum	3.3	5.8	8.9	10.5	12.1	13.7	14.5	14.3	13.0	10.1	5.8	2.9	9.6

[1] Mean of data from 1974 to 1996.

Table 9.2: Rainfall and temperature at Cedara for the 1992/93 and 1993/94 growing seasons

	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Total / Mean
Total Rainfall (mm)													
1992/93	4.3	17.4	16.9	34.2	82.6	68.7	69.2	108.1	11.9	25.0	7.6	0	548.9
1993/94	0.1	27.5	31.66	132.7	68.1	161.5	206.3	126.8	112.8	37.2	15.6	3.5	923.7
Long Term[1] Total	15.3	26.0	48.4	85.2	109.9	128.8	133.5	123.7	112.9	50.5	27.4	15.1	876.7
Mean Temperature (°C)													
1992/93 Maximum	21.4	19.9	22.7	24.2	24.5	26.1	26.7	25.1	25.0	23.8	23.1	19.8	23.5
Minimum	3.5	5.4	10.3	11.1	12.4	15.3	15.3	14.9	13.7	10.7	7.5	3.1	10.3
1993/94 Maximum	21.1	21.0	24.1	21.5	23.9	24.5	24.9	24.4	24.5	22.7	22.0	18.8	22.8
Minimum	5.3	6.0	9.9	12.5	12.6	14.3	14.8	14.4	13.4	11.4	6.7	2.9	10.4
Long Term[2] Mean (°C)													
Maximum	19.2	20.9	22.3	22.6	23.5	24.7	25.0	25.0	24.3	22.7	20.8	19.0	22.5
Minimum	3.7	5.8	8.7	10.7	12.3	13.7	14.8	14.8	13.6	10.6	6.7	3.7	9.9

[1] Mean of data from 1914 to 1996.

[2] Mean of data from 1917 to 1996.

Fungicides were first applied two weeks before flowering. Systemic fungicides were applied three times at 14 day intervals. Protectant fungicides were applied five times at 7 day intervals. The number and concentrations of fungicides applied are shown in Table 9.3. Fungicides were applied at 2 bar pressure in 400ℓ ha^{-1} by knapsack sprayer. Carboxin was a granulated fungicide (Vitavax 4G) which was applied to the soil at planting in 1993/94.

Table 9.3 Fungicide application rates as grams active ingredient ha^{-1} per application for all trials in 1992/93 and 1993/94 seasons

Treatment	Cedara		Greytown	
	1992/93 Rates	1992/93 & 1993/94	1992/93	1993/94
Benomyl	250	375	250	250
Carbendazim		382.5	250	250
Carbendazim/difenoconazole		125/62.5		
Carbendazim/flutriafol		187.5/117.5	150/90	150/90
Carbendazim/flusilazole	250/125	187.5/93.8	250/125	250/125
Carboxin				80
Chlorothalonil			1000	1000
Copper Exp.		85.4		85.4
Cupric hydroxide				1540
Difenoconazole	75	87.5	75	75
Exp. 1[1]				Unknown
Exp. 2[1]				Unknown
Exp. 3[1]		Unknown		
Fluazinam				175
Flusilazole/carbendazim		250/125	250/125	250/125
Flusilazole	125			125
Flutriafol	125	156.25	125	125
Iprodione SC			500	500
Iprodione WP				500
Mancozeb			1600	1600
Oxycarboxin				400
Propiconazole		250	250	250
Tebuconazole		250	250	250
Thiophanate methyl				480
Vinclozolin			250	250

[1] These were experimental fungicides and the chemical companies did not release the names of the active ingredients.

In 1992/93, the trial was assessed for GLS and common rust (caused by *Puccinia sorghi* Schw.) 90 and 125 days after planting, and the days from planting to 80% dry husks was determined. In 1993/94, the trial was assessed 95, 113 and 133 days after planting for GLS and northern corn leaf blight (NCLB - caused by *Exserohilum turcicum* [Pass.] Leonard & Suggs), and the percentage prematurely dead plants determined 145 days after planting. In both years, the trials were assessed for prolificacy and grain yield at harvest.

Cedara

Three trials were conducted at the CADI (29° 31'S, 30° 17'E at an altitude of 1070 m) near Pietermaritzburg. Trials to evaluate various fungicides (Fungicide Evaluation 92/93 and 93/94) were conducted in 1992/93 and 1993/94. A third trial studying the rates of application of selected fungicides (Fungicide Rates 92/93) was conducted in 1992/93. Maize had previously been grown on the sites before the first trials were undertaken in 1992/93. The trials were no-till planted with a John Deere 7000 four-row, Max-Emerge planter at a rate of 50 000 seeds ha^{-1}, to RS 5206 (a GLS-susceptible hybrid). Final plant density was 47 500 plants ha^{-1} in both seasons. Fertilizer sufficient for an eight-ton grain crop ha^{-1} was band-applied at planting. A top-dressing of 100 kg N ha^{-1} was broadcast when maize was knee-high. Normal pest- and weed-control practices for the area were followed. Plots comprised eight, 9.0 m rows spaced 0.75 m apart. The Fungicide Evaluation 92/93 and 93/94 trials were planted in randomised complete blocks designs, replicated three times in 1992/93 and four times in 1993/94. The Fungicide Rates 92/93 trial comprised four replications in a factorial design. The central four-rows of each plot were sprayed with the fungicides, and the central two, 8.0 m rows were hand-harvested.

The Fungicide Rate 92/93 trial was planted on 26 November 1992, and fungicide applications were made 64 and 83 days after planting (DAP). The Fungicide Evaluation 92/93 trial was planted on 26 November 1992 and fungicides applied 76 and 104 DAP. The Fungicide Evaluation 93/94 trial was planted on 17 November 1993 and fungicides applied 72 and 98 DAP. The concentrations of fungicides applied are shown in Table 9.3. The fungicides were applied with a CO_2-pressurised knapsack sprayer fitted with a vertically-mounted spray-boom with three Whirlrain ¼" WRW2-20° nozzles spaced one metre apart. Full cover sprays of 450ℓ ha^{-1} were applied to each maize row.

Percentage leaf area loss due to GLS, based on whole plant standard area diagrams described

Ward *et al.* (1997), were used as a guide in estimating disease severity. Assessments were made regularly on plants in the centre of the two middle-rows of each plot, commencing at first signs of disease until the crop was physiologically mature. Disease was first observed in the trials 69 DAP in 1992/93 and 59 DAP in 1993/94. One thousand kernel weights were made for the rate of fungicide application trial by weighing 1 000 kernels taken from grain samples at 12.5% moisture and were expressed in grams.

At both locations, these data were used in calculating the area under the disease progress curve (AUDPC) using a trapezoidal integration program (Berger, 1981). The AUDPC was standardised (SAUDPC) by dividing the AUDPC by the number of days over which the assessments were under taken. This allowed for more meaningful comparison over locations and between seasons.

In addition, these data were transformed to fit the logistic model (used to estimate the duration of fungicide control). Infection rates were calculated using the formula (Vanderplank, 1963):

$$r=\frac{1}{t}\ln\left(\frac{x}{1-x}\right)$$

From the logistic models, the effective periods of fungicide were calculated. The effective period was determined from the time of fungicide application until the fungicide was no longer effective in controlling the spread of the pathogen.

Grain yields were expressed in t ha^{-1} at 12.5% kernel moisture. The data was analysed using Genstat Version 5.31 (Rothamstead Experimental Station) and data was transformed when appropriate. Statistical analysis of trial data were conducted using an analysis of variance (ANOVA) and mean separations were based on LSD at the 5% level of probability (Greytown) or Duncan's Multiple Range tests (Cedara) of significance. In order to make meaningful comparisons between fungicide groups or types, orthogonal contrasts were made.

A wide range of fungicides were selected for testing at Greytown with the primary objective primarily determining their efficacy against *C. zeae-maydis*, but also against *P. sorghi*, *E. turcicum* and *Stenocarpella* ear rot. At Cedara the number of fungicides per trial were limited, and the fungicide entries were determined by their commercial availability to the farmer for GLS control. In some cases pre-registration products were also included.

9.3 RESULTS

Results are presented in Tables 9.4 - 9.9 and Figures 9.1.

1992/93

Greytown

From Table 9.4 it can be seen there were highly significant differences between infection rates (r) for the different fungicides. Only chlorothalonil and benomyl did not differ significantly from the non-sprayed control. The carbendazim/flutriafol fungicide resulted in the lowest infection rate but ten fungicides were in the same significance group. The carbendazim/triazole combination fungicides had the lowest infection rates. Carbendazim alone was significantly different from the non-sprayed control but not as effective as the triazole and triazole/carbendazim fungicides.

When SAUDPC was used to measure differences in response to fungicide application, all fungicide treatments resulted in significantly less GLS than the non-sprayed control. A number of fungicides, including mancozeb, chlorothalonil and benomyl, were in a least effective group. All triazole fungicides and the carbendazim/triazole combination fungicides were in a most effective group.

All fungicides, except benomyl, resulted in significantly less GLS than the non-sprayed control at Greytown. Chlorothalonil, mancozeb and carbendazim resulted in significantly less GLS than the non-sprayed control but not significantly less than benomyl. Iprodione, benomyl 2N and tebuconazole resulted in significantly less GLS than both the non-sprayed control and benomyl but were not significantly different from the previously mentioned group. Propiconazole, vinclozolin, difenoconazole, difenoconazole 2N, propiconazole 2N and carbendazim/flusilazole resulted in significantly less GLS than the non-sprayed control, benomyl and chlorothalonil, but not significantly different from the other fungicides mentioned previously. The most effective fungicides were those belonging to the triazole and benzimidazole/triazole fungicide groups.

Table 9.4: Grey leaf spot (GLS) (r values, AUDPC and final percentage severity), northern corn leaf blight (NCLB), days to 80% dry husks, prolificacy and grain yield (t ha^{-1}) results for various fungicides applied to PAN 6528 planted in Greytown during 1992/93

Treatment	GLS			% NCLB	Prol	Yield
	Infection rate (x100)	SAUDPC	%GLS			
Tebuconazole 2N	3.93 gh	1.09 e	1.27 fgh	0.71 e	0.526 a	4.09 a
Tebuconazole	8.20 cde	3.85 cde	2.23 cdefgh	0.71 e	0.767 b	6.89 b
non-sprayed control	13.53 a	19.14 a	5.10 a	2.39 a	0.953 cd	7.04 b
Propiconazole 2N	6.50 efg	2.12 e	1.72 efgh	0.71 e	0.956 cd	7.64 bc
Difenoconazole 2N	7.03 defg	2.12 e	1.77 efgh	0.71 e	0.930 cd	8.24 bcd
Chlorothalonil	10.70 abc	7.55 c	3.20 bc	0.80 de	0.951 cd	8.35 bcd
Vinclozolin	8.63 cde	4.09 cde	2.35 def	1.45 b	0.950 cd	8.49 bcd
Mancozeb	9.47 bcde	7.55 c	3.08 bcd	1.08 cd	0.984 cd	8.56 bcd
Benomyl	12.30 ab	11.99 b	4.06 ab	0.73 e	1.016 cd	8.66 bcd
Propiconazole	7.13 defg	2.37 e	1.84 efgh	0.71 e	0.983 cd	8.75 bcd
Difenoconazole	7.87 cdef	2.86 de	2.03 defgh	0.71 e	0.953 cd	8.75 bcd
Carbendazim/flusilazole	7.10 defg	2.12 e	1.72 efgh	0.71 e	0.933 cd	8.84 bcd
Benomyl 2N	9.77 bcd	6.81 cd	2.92 cd	0.71 e	0.950 cd	8.96 cd
Flutriafol	4.40 gh	0.90 e	1.15 gh	0.71 e	0.886 bcd	8.98 cd
Iprodione	9.50 bcde	5.08 cd	2.64 cde	1.56 b	1.017 cd	9.04 cd
Carbendazim	10.07 bcd	6.81 cd	2.98 bcd	0.71 e	0.982 cd	9.16 cd
Flusilazole/carbendazim	5.03 fgh	1.13 e	1.34 fgh	0.71 e	1.070 d	9.36 cd
Carbendazim/flutriafol	2.93 h	0.59 e	1.05 h	0.73 e	0.900 bc	9.37 cd
Flusilazole/carbendazim 2N	4.80 fgh	1.09 e	1.29 fgh	0.71 e	0.929 cd	9.46 cd
Flusilazole	4.40 gh	0.89 e	1.22 gh	0.71 e	1.018 cd	9.65 d
Mean	7.67	4.51	2.24	0.90	0.933	8.41
$L.S.D._{0.05}$	3.44	4.33	1.08	0.30	0.150	1.98
%C.V.	27.1	58.1	29.1	20.5	9.8	14.1

AUDPC - area under the disease progress curve
%GLS - percentage leaf area lost due to *C. zeae-maydis* ($\sqrt{(x+0.5)}$ transformation).
%NCLB - percentage leaf area lost due to northern corn leaf blight - *E. turcicum* ($\sqrt{(x+0.5)}$ transformation).
Prol - number of ears per plant.
Yield - grain yield in t ha^{-1}

Means separation by L.S.D.. Means with the same letter do not differ at the $p \leq 0.05$ level.

Table 9.5: Grey leaf spot disease severity, infection rate, AUDPC values, effective period of control and grain yield (t ha^{-1}) for various fungicides during the 1992/93 and 1993/94 seasons at Cedara

Treatment	Grey leaf spot								Grain yield		
	%GLS[1]		Infection rate (r x 100)		SAUDPC[2]		Effective period of control [3] (days)		% Crude Protein	Grain yield	
	92/93[4]	93/94	92/93	93/94	92/93	93/94	92/93	93/94	92/93	92/93	93/94
non-sprayed control	83.7 a	90.0 a	16.52 a	10.90 a	41.78 a	51.71 a	0.00 a	0.00 a	9.995 a	4.227 a	4.858 a
Benomyl	18.1 cde	28.1 c	6.90 bc	5.87 d	8.34 de	9.15 e	21.00 cd	18.00 bc	9.313 c	7.319 c	6.964 b
Difenoconazole	32.5 c	53.8 b	7.37 bc	7.45 b	15.79 c	18.51 c	10.00 b	14.00 b	9.775 ab	7.731 c	7.205 b
Flutriafol	12.5 de	34.7 c	6.87 bc	6.57 bcd	8.11 def	12.27 de	13.50 bc	18.50 bcd	9.620 abc	7.723 c	6.995 b
Carbendazim/flutriafol	7.6 de	33.1 c	6.35 bc	7.25 bc	6.50 ef	9.00 e	28.25 de	21.00 bcd	9.485 bc	7.568 c	7.810 b
Carbendazim/flusilazole	4.2 e	26.2 c	4.30 c	6.07 cd	4.74 f	9.72 e	30.75 e	26.00 de	9.502 bc	7.727 c	7.411 b
Flusilazole/carbendazim	8.9 de		5.57 cc		6.90 ef		22.75 de		9.575 bc	6.882 bc	
Propiconazole	23.1 cd		7.20 bc		11.10 d		20.50 cd		9.743 ab	7.580 c	
Tebuconazole	53.7 b		9.70 b		25.29 b		3.00 ab		9.788 ab	5.913 b	
Flusilazole	11.9 cde		6.37 bc		8.64 de		25.25 de		9.438 bc	7.948 c	
Exp. 3		35.0 c		7.20 bc		12.38 de		14.50 b			7.050 b
Copper exp.		87.5 a		11.50 a		39.39 b		5.75 a			4.482 a
Carbendazim/difenoconazole		25.6 c		6.32 bcd		10.13 e		25.25 cde			7.561 b
Carbendazim		28.8 c		6.65 bcd		7.18 e		29.75 e			7.456 b
Mean	25.6	44.2	7.72	7.58	13.72	17.94	17.50	17.27	9.623	7.062	6.874
L.S.D. (0.05)	17.1	13.3	3.26	1.24	3.57	5.87	9.10	7.55	0.411	1.274	1.348
%CV	32.4	14.6	29.1	11.3	17.9	22.6	35.8	30.1	2.1	8.8	9.7

[1] Final disease severity is percentage leaf-blighting near physiological maturity.

[2] SAUDPC - standardised area under disease progress curve.

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

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

Means separation by L.S.D.. Means with the same letter do not differ at the $p \leq 0.05$ level.

Table 9.6: The infection rate (r values), AUDPC values, effective period of control and grain yield (t ha^{-1}) for different rates of application for different fungicides during the 1992/93 season at Cedara

Treatment	Infection Rate (r x100)				AUDPC				GLS			
	0.5	1	2 [1]	Mean	0.5	1	2 [1]	Mean	0.5	1	2 [1]	Mean
non-sprayed control	9.07	9.07	8.73	8.96 a	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
L.S.D.$_{0.05}$ between fungicides	1.05				197				7.9			
L.S.D.$_{0.05}$ between rates	0.69				129				5.2			
L.S.D.$_{0.05}$ within rates and fungicides	2.05				384				15.0			
%C.V.	17.7				23.7				36.6			

Notes:

[1] Rates at which fungicides were applied (see Table 9.3): 0.5 = half the recommended rate
1 = the recommended rate
2 = double the recommended rate

[2] r values = logistic rate of increase in GLS x100.

Means separation by L.S.D.. Means with the same letter do not differ at the $p \leq 0.05$ level.

Table 9.7: The effective period of control and grain yield (t ha^{-1}) for different rates of application for different fungicides during the 1992/93 season at Cedara

Treatment	Effective Period of Control (days)				Grain Yield			
	0.5	1	2 [1]	Mean	0.5	1	2 [1]	Mean
non-sprayed control	0.00	0.00	0.00	0.00 a	5.889	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	62.33	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	7.374 a	8.238 b	8.261 b	7.959
L.S.D.$_{0.05}$ between fungicides	5.55				0.771			
L.S.D.$_{0.05}$ between rates	3.64				0.506			
L.S.D.$_{0.05}$ within rates and fungicides	10.83				1.504			
%C.V.	9.9				9.1			

Notes:

[1] Rates at which fungicides were applied (see Table 9.3): 0.5 = half the recommended rate
1 = the recommended rate
2 = double the recommended rate

Means separation by L.S.D.. Means with the same letter do not differ at the $p \leq 0.05$ level.

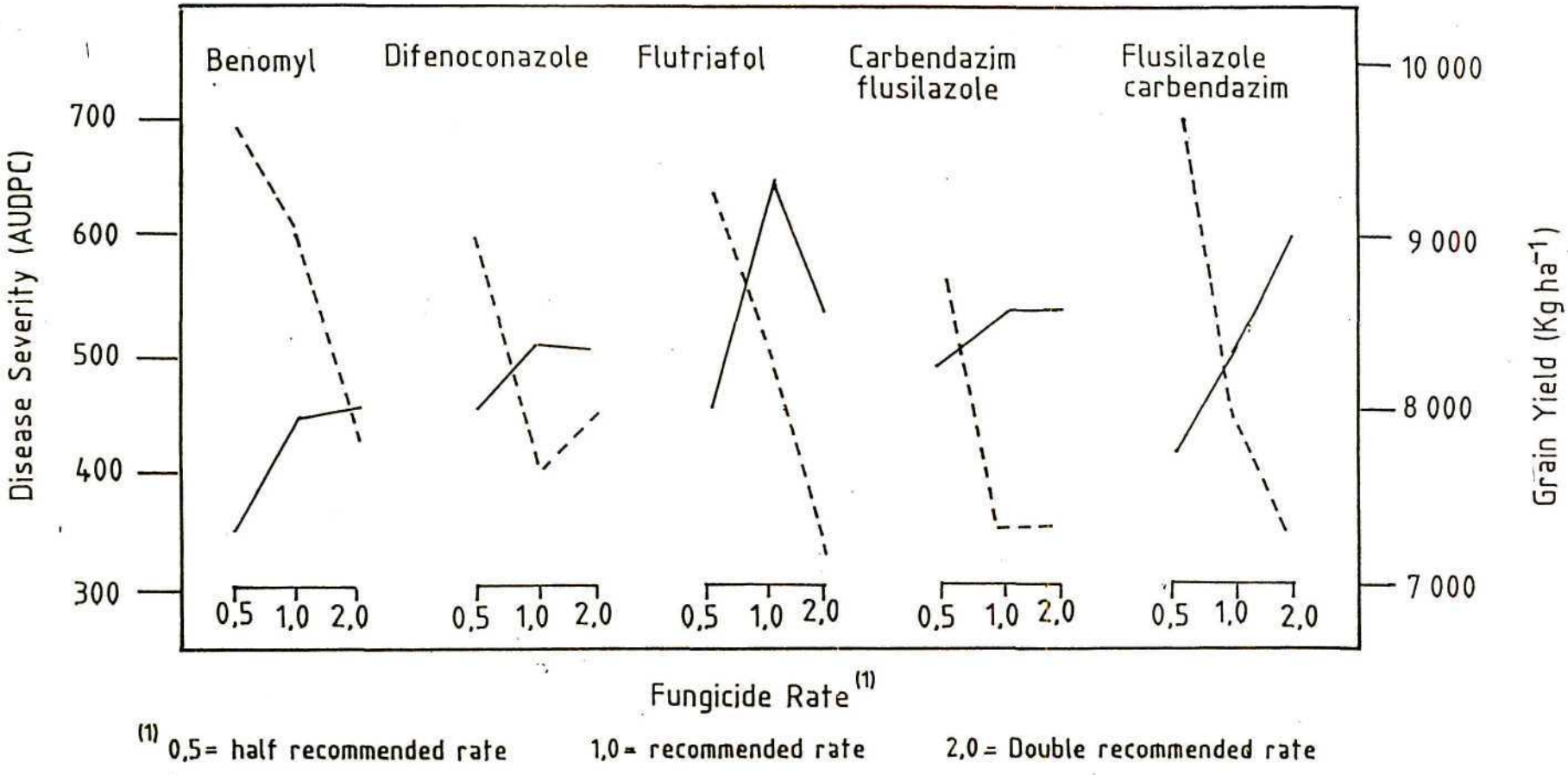

Figure 9.1: The effect of rate of application of different fungicides on disease severity (- - -) and grain yield (kg ha^{-1}) (_____)

Although the levels of northern corn leaf blight (NCLB) were not as high as the GLS, all fungicides significantly reduced the %NCLB when compared to the non-sprayed control. Of the fungicides, iprodione and vinclozolin (dicarboximide group of fungicides) were the least effective in controlling NCLB. Benomyl, chlorothalonil, mancozeb and carbendazim/ flutriafol controlled NCLB significantly better than the dicarboximides. Only mancozeb was significantly less effective in controlling NCLB than all the remaining fungicides, which were not significantly different from each other.

Maize treated with tebuconazole 2N was significantly less prolific than all other treatments. Tebuconazole-treated maize only had 0.767 ears plant^{-1} but was not significantly different from the flutriafol and carbendazim/flutriafol treatments.

Tebuconazole 2N resulted a significantly lower grain yield than all other treatments. The non-sprayed control did have a low grain yield but was not significantly different from a large number of fungicide treatments, including tebuconazole. Benomyl 2N, flutriafol, iprodione, carbendazim, carbendazim/flutriafol, flusilazole/carbendazim, flusilazole and flusilazole/ carbendazim 2N resulted in a significantly greater grain yield than the non-sprayed control. The trend was clear that systemic fungicide treatments, except for tebuconazole, resulted in a higher grain yield than the non-sprayed control. Although application of protectant fungicides to maize resulted in increase in grain yields, they were not significantly different from the non-sprayed control.

Cedara

Comparison of the infection rates (Table 9.5) show that all fungicides significantly reduced the rate of increase of the epidemic. However, there were no significant differences between the fungicides.

From the SAUDPC values (Table 9.5), it is apparent that all fungicides resulted in significantly less GLS than the non-sprayed control, the least effective being tebuconazole. The most effective treatments were flutriafol and the triazole/benzimidazole combination fungicides.

From Table 9.5 it can be seen that all fungicide treatments significantly reduced the severity of GLS. Of these fungicides, the least effective were tebuconazole and difenoconazole. The

most effective treatments were the triazole/benzimidazole combination fungicides.

The percentage crude protein was determined (Table 9.5) and benomyl, flusilazole and the triazole/carbendazim fungicides were found to significantly reduce the amount of crude protein compared to the non-sprayed control. The lowest percentage crude protein was recorded for the benomyl treatment, which did not differ significantly from flusilazole and the triazole/benzimidazole combination fungicide treatments.

All fungicides resulted in a significant increase in grain yield (Table 9.5) when compared to the non-sprayed control. The least effective treatments were the flusilazole/carbendazim and tebuconazole treatments. Only the tebuconazole treatment had significantly less grain yield than the other fungicides, except for flusilazole/carbendazim.

Infection rates in the Fungicide Rates 92/93 trial were all significantly lower for all fungicides compared to the non-sprayed control (Table 9.6 and Figure 9.1). There were no significant differences between fungicides. However, both the mean for the 1N and 2N rates of fungicides had significantly lower infection rates than did the mean for the 0.5N rates. There were significant differences in infection rates between the 0.5N and 1N rates for the fungicides, but not for benomyl and flutriafol. There were no significant differences in control between the means for the 1N and 2N rates, with the flutriafol and flusilazole/carbendazim treatments being most effective at the 2N rates.

The AUDPC values resulted in similar trends as for the infection rates (Tables 9.6).

The GLS readings (Table 9.6) showed all fungicides to significantly reduce GLS to levels below that of the non-sprayed control. The least effective fungicide in controlling the GLS was difenoconazole, which was significantly worse than flutriafol only. The other fungicides did not show significant differences amongst themselves. In all cases, except flusilazole/carbendazim, the increase in fungicide rates from 0.5N to 1N resulted in significantly less GLS. Benomyl, flusilazole/carbendazim and difenoconazole were significantly more effective at the 2N rates.

The effective period of control showed that there are significant differences in efficacy between fungicides (Table 9.7). The longest period of control was obtained by the

application of carbendazim/flusilazole, and this treatment was significantly better than the benomyl treatment. A common trend was that increased rates of fungicides resulted in increased periods of control, especially for flusilazole/carbendazim. The effective period of control for the 0.5N rate was significantly smaller than for the 1N rate at the 7% level of significance. The 2N rate resulted in significantly less GLS than the 0.5N rate. The rate at which difenoconazole and carbendazim/flusilazole was applied did not have a significant effect on the duration of control.

All the fungicides applied, and at all rates, resulted in a highly significant increase in grain yield (Table 9.7). The least effective of the fungicides was benomyl but the grain yield differed significantly only from difenoconazole. Grain yield increased from 31.3% - 48.3% for the various fungicides when compared to the non-sprayed control. There was a significant increase in grain yield when the rates were increase from 0.5N to 1N but no further increase occurred when the rates were increased to 2N.

1993/94

Greytown

The more conducive weather conditions resulted in higher infection rates than for the 1992/93 season (Table 9.8). The infection rate for the carboxin treatments was not significantly different than that for the non-sprayed control. Although the copper fungicides had significantly lower infection rates than the non-sprayed control, the infection rates were significantly higher than for all other treatments, except iprodione WP and oxycarboxin. All the treatments containing either a triazole or benzimidazole, or the combination, resulted in a significantly lower infection rate than the other treatments. The triazole/benzimidazole combination fungicides resulted in the lowest infection rates. The protectant fungicides, chlorothalonil and mancozeb, resulted in a significantly lower infection rate than the non-sprayed control and some of the more systemic fungicides. Iprodione SC resulted in a significantly lower infection rate than did the iprodione WP treatment.

Table 9.8: Grey leaf spot (GLS) (r values, AUDPC and final percentage severity), common rust, percentage prematurely dead plants, prolificacy and grain yield (t ha^{-1}) results for various fungicides applied to PNF 6552 planted in Greytown during 1993/94

Treatment	GLS			%Ps	PreD	Prol	Yield
	Infection rate (x100)	SAUDPC	%GLS				
Cupric hydroxide	13.25 b	11.67 bc	6.254 ab	6.212 a	8.23 a	1.035	3.940 a
Copper Exp.	13.33 b	13.73 b	6.063 abc	5.372 bc	6.13 ab	0.996	5.002 ab
Fluazinam	11.52 cde	4.62 fg	5.155 defg	4.736 cd	3.34 de	1.1101	5.473 bc
Chlorothalonil	10.83 de	7.33 de	5.607 bcde	4.943 bcd	3.65 cde	0.992	5.498 bc
Iprodione SC	10.18 e	4.09 fgh	4.991 efgh	5.109 bcd	4.03 bcde	0.992	5.581 bc
Carboxin	15.33 a	18.08 a	6.519 a	5.304 bcd	5.98 ab	1.099	5.630 bc
non-sprayed control	15.28 a	17.50 a	6.485 a	5.480 b	5.87 bc	1.090	5.653 bc
Vinclozolin	11.15 d	6.19 ef	5.441 cdef	4.973 bcd	4.51 bcd	1.136	5.719 bc
Oxycarboxin	12.10 bcd	7.20 de	5.578 bcde	4.963 bcd	4.24 bcd	1.091	6.029 bc
Mancozeb	10.65 cde	7.37 de	5.441 cdef	4.772 cd	2.87 de	1.070	6.061 bc
Propiconazole	6.57 gh	1.68 hij	4.126 ijkl	4.740 cd	2.30 de	1.063	6.099 bc
Exp. 2	5.82 ghij	1.30 ij	3.859 jkl	5.047 cd	3.38 de	1.007	6.100 bc
Thiophanate methyl	5.57 ghij	1.17 ij	3.727 jklm	4.879 bcd	4.01 bcde	1.140	6.296 bc
Flusilazole/carbendazim	4.65 jk	0.98 ij	3.582 klm	4.804 cd	3.91 bcde	1.111	6.344 bc
Carbendazim/flutriafol	4.65 jk	0.98 ij	3.582 klm	4.802 cd	1.75 e	1.097	6.429 bcd
Difenoconazole	6.55 gh	1.81 hij	4.223 ijk	4.846 cd	2.49 de	1.100	6.506 cd
Tebuconazole	8.27 f	3.15 ghi	4.779 fghi	4.927 bcd	3.45 de	1.233	6.536 cd
Iprodione WP	12.83 bc	9.45 cd	5.854 abcd	5.078 bcd	3.64 cde	1.086	6.564 cd
Exp.1	4.70 ijk	0.91 ij	3.380 lm	4.922 bcd	1.80 e	1.155	6.632 cd
Flusilazole	6.32 ghi	1.64 hij	3.994 jkl	4.966 bcd	1.92 e	1.086	6.646 cd
Carbendazim	6.10 ghij	1.62 hij	4.055 ijkl	5.017 bcd	2.79 de	1.128	6.724 cd
Benomyl	7.03 fg	2.19 ghij	4.409 ghij	4.859 bcd	3.15 de	1.039	6.815 cd
Carbendazim/flusilazole	3.52 k	0.58 j	3.060 m	4.695 cd	2.57 de	1.128	6.939 cd
Flutriafol	4.93 hijk	1.04 ij	3.651 jklm	4.677 d	3.91 bcde	1.262	7.848 d
Mean	8.80	5.26	4.741	5.005	3.75	1.093	6.128
$L.S.D._{0.05}$	1.65	2.54	0.766	0.617	2.30	n.s.	1.480
%C.V.	13.0	34.3	8.1	6.3	30.8	12.5	12.1

AUDPC - area under the disease progress curve
%GLS - AUDPC for *C. zeae-maydis* (log(x+0.5) transformation).
%Ps - AUDPC for common rust - *P. sorghi* (log(x+0.5) transformation).
PreD - percentage plants pre-maturely dead at 140 after planting ($\sqrt{}$(x+0.5) transformation).
Prol - number of ears per plant.
Yield - grain yield in t ha^{-1} (corrected for plant stand).
Means separation by L.S.D.. Means with the same letter do not differ at the $P \leq 0.05$ level.

The SAUDPC values showed that the non-sprayed control and carboxin treatments were not significantly different from each other but had significantly more GLS than did all other treatments. The copper fungicides were the least effective of the foliar fungicide treatments. All the treatments containing either a triazole or benzimidazole, or the combination resulted in a significantly lower infection rate than the other treatments. The triazole/benzimidazole combination fungicides resulted in the lowest infection rates. Iprodione SC resulted in a significantly lower SAUDPC value than did the iprodione WP treatment.

When GLS severity is examined, the non-sprayed control, carboxin, cupric hydroxide, copper exp. and iprodione WP were not significantly different from each other. Chlorothalonil, oxycarboxin, vinclozolin and mancozeb reduced the GLS significantly when compared to the non-sprayed control. However, these treatments were not different from the copper exp. and iprodione WP treatments. Iprodione SC resulted in significantly less GLS than iprodione WP. The most effective fungicides, which were not significantly more effective than each other, were thiophanate methyl, flutriafol, carbendazim/flutriafol, flusilazole/carbendazim, exp. 1, and carbendazim/flusilazole. The most effective of these treatment was carbendazim/ flusilazole.

The application of cupric hydroxide significantly increased the incidence of common rust when compared to the non-sprayed control. Most of the protectants and some of the systemic fungicides were not significantly different from the non-sprayed control. Mancozeb, fluazinam, difenoconazole, propiconazole, Exp. 2, flutriafol, carbendazim/flutriafol, flusilazole/carbendazim and carbendazim/flusilazole all resulted in significantly less common rust than the non-sprayed control. The most effective treatment in controlling common rust was flutriafol, although it was not significantly different from a number of the other triazole and triazole/carbendazim combinations.

Both copper fungicides increased the number of prematurely dead plants when compared to the non-sprayed control, although only cupric hydroxide caused significantly more. The most effective treatments in reducing premature death were flusilazole, carbendazim/flutriafol and exp. 1. However, these treatments were not significantly better than a number of other fungicides, including mancozeb and chlorothalonil.

The prolificacy of the hybrids was not significantly affected by the application of fungicides.

Although there were significant differences in grain yield, the differences between fungicide treatments and the non-sprayed control were seldom significant (Table 9.5). The application of copper fungicides to PNF 6552 resulted in a significantly reduced grain yield, particularly when cupric hydroxide was applied. Difenoconazole, tebuconazole, iprodione WP, exp. 1, flusilazole, carbendazim, benomyl, carbendazim/flusilazole and flutriafol all resulted in significant increased grain yield when compared to the non-sprayed control, flutriafol being the most effective.

Cedara

The infection rates of the fungicide treatments were all significantly lower than for the non-sprayed control and the copper fungicide (Figure 9.2 and Table 9.6). The least effective treatments in reducing the infection rate were difenoconazole and Exp. 3.

The SAUDPC values (Table 9.5) showed that all treatments significantly reduced the amount of GLS present over time. The copper exp. fungicide controlled GLS significantly less than all the other fungicide treatments. Of the triazole and benzimidazole fungicides, difenoconazole was the least effective.

Table 9.5 shows that most fungicides significantly reduced GLS levels when compared to the non-sprayed control. The copper exp. fungicide did not differ significantly from the non-sprayed control. The least effective of the fungicides was difenoconazole, which resulted in significantly more GLS than did all other treatments. The remainder of the fungicides were equally effective.

The effective period of control differed significantly between fungicide treatments. The copper exp. fungicide was not significantly different from the non-sprayed control. Difenoconazole only resulted in 14 days of GLS control. The three most effective fungicides were carbendazim/difenoconazole, carbendazim/flusilazole and carbendazim with 25, 26 and 30 days control, respectively.

All fungicides, except copper exp., resulted in significantly increased grain yields compared to the non-sprayed control. Differences in grain yield between the remainder of the fungicides were non-significant.

9.3 DISCUSSION

The 1992/93 growing seasons was characterised by unusually low rainfall, with hot days (Tables 9.1 and 9.2). It was only in mid- to late grainfill that rainfall normalised. Greytown received significantly more rainfall in 1992/93 than did Cedara. However, the GLS epidemic was still very late in materialising. In contrast, rainfall during the 1993/94 season was normal at Cedara, above average in the early season at Greytown and well distributed throughout the growing season. Cedara in 1993/94 received 185.2mm more rain than did Greytown. Mists were abundant, especially in January and February. Temperatures were lower than average at Cedara. The overall disease severity in the drier 1992/93 season was significantly lower than in 1993/94. From the maximum and minimum temperatures in Tables 9.1 and 9.2, Cedara is generally slightly cooler than Greytown. This could account for the fact that the GLS epidemics usually start earlier and are more severe at Cedara than Greytown.

Most fungicide treatments across both sites and seasons significantly reduced levels of GLS leaf-blighting when compared to the non-sprayed control (Tables 9.4 - 9.5 and 9.8), the main exceptions being copper fungicides and a carboxin granular fungicide applied at planting. Overall, the fungicide mixtures of carbendazim and triazole fungicides provided significantly better control of GLS leaf-blighting than single-product triazole or benzimidazole fungicides. However, this trend was not consistent in all seasons and at both sites (Table 9.9), although it was more consistent at Greytown. It is noteworthy that the combination fungicides resulted in less GLS than the single product fungicides, but this benefit was not translated into significant grain yield increases. Therefore, there is no benefit to the farmer in using fungicide mixtures, other than to reduce the likelihood of resistance building up to the fungicides in the pathogen. In general, the triazole fungicides were more effective in controlling GLS than the benzimidazole fungicides (Table 9.4 - 9.8).

Table 9.9: Significance levels of orthogonal contrasts for the comparison of various fungicide classes at both Greytown and Cedara during 1992/93 and 1993/94

Comparison	Variate							
	r	SAUDPC	%GLS	Control	%Et	%Ps	Prol	PreD	Yield
Greytown 1992/93									
Benz vs Comb	≤0.001	≤0.001	n.s.		≤0.001		n.s.		n.s.
Triazoles vs Comb	n.s.	n.s.	n.s.		n.s.		n.s.		n.s.
Single vs Comb	0.002	0.007	n.s.		0.002		n.s.		n.s.
1N vs 2N	0.068	0.031	n.s.		0.041		0.005		0.058
V/I vs M/C	n.s.	0.057	≤0.001		n.s.		n.s.		n.s.
Prot vs Systemic	n.s.	0.057	≤0.001		≤0.001		n.s.		n.s.
Cedara 1992/93									
Single vs Comb	0.018	≤0.001		≤0.001					
Benz vs Comb	n.s.	n.s.		n.s.					
Triazoles vs Comb	0.017	≤0.001		≤0.001					
Greytown 1993/94									
Benz vs Comb	≤0.001	n.s.	≤0.001			n.s.	n.s.	n.s.	n.s.
Triazoles vs Comb	≤0.001	n.s.	≤0.001			n.s.	n.s.	n.s.	n.s.
Single vs Comb	≤0.001	n.s.	≤0.001			n.s.	n.s.	n.s.	n.s.
Cu vs O Prot	≤0.001	≤0.001	≤0.001			≤0.001	n.s.	≤0.001	≤0.001
V/I vs M/C	n.s.	n.s.	n.s.			n.s.	n.s.	n.s.	n.s.
Prot vs Systemic	≤0.001	≤0.001	≤0.001			≤0.001	0.025	≤0.001	≤0.001
Cedara 1993/94									
Single vs Comb	n.s.	n.s.		n.s.					
Benz vs Comb	n.s.	n.s.		n.s.					
Triazoles vs Comb	n.s.	0.007		0.023					

r = rate of increase of GLS
SAUDPC = standardised area under the disease progress curve
%GLS = % leaf area loss due to GLS
Control = days control resulting from fungicide application
%Et = % leaf area loss due to *E. turcicum*
%Ps = % leaf area loss due to *P. sorghi*
Prol = number of ears per plant
Yield = grain yield
Benz = benzimidazole fungicides
Comb = fungicides with more than one active ingredient
1N = the normal recommended rate of fungicide
2N = twice the normal recommended concentration
V/I = vinclozolin and iprodione
M/C = mancozeb and chlorothalonil
Cu = copper fungicides
Prot = protectant fungicides
O Prot = protectant fungicides other than copper

In general, the protectant fungicides did not give as good control of GLS leaf-blighting as systemic fungicides (Table 9.9), but did significantly reduce the amount of GLS compared to the non-sprayed control at Greytown. This difference was also translated into a grain

yield benefit during the 1993/94 season. In the USA, it was found that chlorothalonil, at 5.03kg a.i. ha^{-1}, could induce phytotoxicity under conditions of drought stress, and copper thallate consistently resulted in phytotoxicity both in the presence and absence of GLS. The toxicity response was not hybrid specific (Rivera-Canales, 1993; Martinson and Munkvold, 1995). In contrast, phytotoxicity caused by chlorothalonil was not observed under drought conditions in South Africa, but severe phytotoxicity due to the application of copper fungicides was observed consistently. Not only did copper fungicides have little effect upon the incidence of GLS, but also significantly increased the incidence of common rust. This was possibly occurred as a result of the stress the plants experienced. The proportion of premature dead plants also increased significantly upon the application of copper fungicides, primarily the copper exp. fungicide.

In 1993/94, there was a significant difference between the efficacy of iprodione SC and iprodione WP, with the former giving better GLS control but a lower yield increase. It is possible that the SC formulation results in better uptake of the fungicide, resulting in improved GLS control but also has a slight phytotoxic effect on the plant. A good combination fungicide could be carbendazim and iprodione formulated as an SC. When the efficacy of the dicarboximide fungicides were compared to that of mancozeb and chlorothalonil at Greytown (see Table 9.9), it was only in the 1992/93 season that the dicarboximides controlled the incidence of GLS significantly better, but with no yield benefit.

SAUDPC, AUDPC and final-disease severity were excellent parameters for evaluating fungicide performance. These values, however, did not always relate directly to the grain yields harvested. In this respect, the infection rate, with less definitive differences between treatments, correlated more closely with the grain yield. However, there were cases were the GLS blight was poorly controlled but yields were very good. Difenoconazole-treated maize, for instance, had the worst GLS leaf-blighting of the triazole-treated plots, yet surprisingly, produced among the highest grain yields. Benomyl, on the other hand, provided among the best control of GLS (except Greytown in 1992/93 - Table 9.4), but generally had the lowest grain yield. It is possible that these anomalies may be due to the different growth-regulating properties of the fungicides evaluated. The triazole fungicides act by sterol-inhibition and have plant-growth regulating properties (Lonsdale and Kotze, 1993). In contrast, benomyl has been shown to have no effect on maize growth when applied alone, but in combination with chlorothalonil, reduced the number of internodes

(Smith, 1989). A more likely explanation, however, is that triazoles control a broader spectrum of diseases, such as common rust and NCLB, than do benzimidazoles. Common rust was present at varying levels in all the trials conducted over both sites and seasons. It would be of interest to determine the effects of the various fungicides on stalk rotting fungi. In the absence of leaf diseases, the application of triazole and dicarboximide fungicides can result in plants staying greener for up to 21 days longer than the non-sprayed controls (Nowell, unpublished). This is referred to as the "tonic effect" by Zadoks and Schein (1979).

At Cedara, the response of GLS to the three rates of fungicides applied was significant, with the 0.5N rate being less effective than the higher rates applied. Figure 9.1 shows a trend for yields to "flatten" with the application of recommended rates and double rates, except for flusilazole/carbendazim and carbendazim/flusilazole. Flusilazole/carbendazim provided maximum GLS control and grain yield at the highest rate. In 1992/93 at Greytown, some fungicides were applied at both the recommended rate and at double the concentration (2N). Although, all 2N treatments resulted in less GLS, propiconazole and difenoconazole at 2N resulted in significantly less grain yield than the recommended rates of fungicide treatments. In addition, tebuconazole, at both 1N and 2N, resulted in less grain yield than the non-sprayed control. This suggests that under drought conditions, these products can result in phytotoxicity, particularly tebuconazole. This needs to be investigated further before definite conclusions can be made.

The longest-duration of control was achieved in the drier 1992/93 season, which was less favourable for disease. Overall, fungicide mixtures provided the longest periods of control of all the fungicides evaluated. This parameter emphasised the poor control difenoconazole alone provided, with GLS control lasting only 10 - 14 days. However, when combined with carbendazim, this product gave the longest effective control of GLS.

Analysis of yield results indicate the GLS may cause yield decreases of greater than 50%. In 1993/94 at Greytown, yield increases of 41.1% (significantly different treatment only) resulted when fungicides were applied to a susceptible commercial hybrid. In 1992/93, the yield increases were 68.5 - 135.9% but this was on a sister line single hybrid used in seed production. This also partially accounts for the lower yield potential compared to the previous season. Only three applications of systemic fungicides, starting two weeks before

flowering, were applied at Greytown, regardless of the severity of the epidemic. This was to simulate the actual maximum spray programmes in seed production fields. For this reason, the fungicides did not adequately control the late epidemic and yield differences were minimised. At Cedara, the yield increase due to fungicide application was 43.4 - 60.7% in 1993/94 and 62.8 to 88.0% in 1992/93 (significantly different treatments only).

When fungicides were applied to maize with GLS, the total crude protein yield ha^{-1} was increased significantly. However, the proportion per unit mass decreased compared to an non-sprayed control. For example, the benomyl treatment resulted in the lowest crude protein content at Cedara in 1992/93, 6.82% less than the non-sprayed control. This change may be significant in determining a balanced feed mix for animals and needs to be investigated in more detail, as will the effect of fungicide residues in animal feeds, especially with the long life / persistent triazole group of fungicides.

There was a good correlation between sites with respect to fungicide response, control of GLS and grain yield. The exceptions to this were benomyl, carbendazim and flutriafol. However, this can be explained when the concentrations used are examined in Table 9.3. The rate of both benomyl and carbendazim was higher at Cedara in both seasons compared to that at Greytown. Flutriafol and carbendazim/flutriafol were applied at a rate about 30% higher at Cedara than at Greytown. This would account for the slightly better performance at Cedara than Greytown. The rate of carbendazim/flusilazole was 25% lower at Cedara than at Greytown. However, this did not apparently significantly affect the results (Tables 9.6 and 9.7).

In the USA, much research has been undertaken to determine the efficacy of fungicides in controlling GLS. The most effective fungicides in USA trials, when severe GLS epidemics occurred, were benomyl, propiconazole, flusilazole and tebuconazole. For effective control of GLS, at least two application of systemic fungicides were applied. The protectant fungicides mancozeb, maneb-zinc, chlorothalonil and copper thallate were less effective, particularly under high inoculum pressure (Hilty *et al.*, 1979; Ayres *et al.*, 1985; Smith, 1989; Stromberg, 1990; Lipps and Pratt, 1991; Stromberg and Carter, 1991; Carter, 1992; Carter and Stromberg, 1992a and 1992b; Rivera-Canales, 1993; Stromberg and Flinchum, 1993; Ward *et* al., 1993; Martinson *et al.*, 1994; Wegulo, 1994; Martinson and Munkvold, 1995; Ward and Nowell, 1997). Protectant fungicides have been associated with a significant

reduction in GLS-blighted leaf area but frequently there has been no corresponding significant increase in grain yield (Hilty *et al.*, 1979; Ayres *et al.*, 1985; Martinson and Munkvold, 1995). These results are in close agreement with the general trends found in South Africa. Where chlorothalonil was applied before quantifiable amounts of GLS was present, it was able to effectively control GLS and resulted in a significant grain yield and seed increase. In these trials, chlorothalonil was more effective in controlling GLS than propiconazole (Rivera-Canales, 1993). This is an option that needs further investigation under South African conditions as fungicides are normally applied once the lower five leaves are showing GLS lesions. An early application of a protectant fungicide may save one application of a systemic fungicide. This would translate into a significant economic benefit. These results are similar to the results of fungicide trials to control *Ascochyta pinodes* (Berk. & Blox.) Vestergr. on peas in South Africa, where preventative sprays of chlorothalonil were more effective and far more cost effective than any systemic fungicide (van Schoor, 1990).

Chemical control measures in the USA are not widely recommended as these are usually uneconomical except for seed producers (Ringer and Grybauskas, 1995). Many factors affect a decision to apply a fungicide to control GLS, with the most important ones being:

i) the growth stage that the epidemic starts,
ii) the germplasm planted (resistance level), and
iii) the prevailing weather conditions.

Before using fungicides to control GLS, it is important to determine the economic feasibility of such applications. Net returns vary within and between seasons, affected by climatic conditions, timing of fungicide application and the number of applications. Greatest economic benefit resulted from the application of fungicides when high levels of GLS were present. The cost:benefit ratio needs to be investigated under South African conditions over a number of seasons and sites. These data from KwaZulu-Natal should not be extrapolated into the main maize production regions of South Africa where production costs, yield potential and climate are significantly different. Only registered fungicides should be applied at the recommended rates.

Bair and Ayres (1986) noted significant variability in the natural *C. zeae-maydis* isolates collected in the fields and suggested that this could mean that resistance to single site systemic fungicides could arise relatively rapidly. Cases of pathogen populations developing resistance to fungicides, especially to the benzimidazole group of fungicides, have become

common in many crops, and in some cases they have developed rapidly. It was decided that a dual active ingredient policy would be followed with commercial products to reduce the risks of resistance building up in the fungus to any single active ingredient. This is in line with proposals for resistance strategies (Delp, 1980; Delp, 1984; Staub and Sozzi, 1984).

The current fungicides have already been used on a commercial scale over most of KwaZulu-Natal for four growing seasons. To preserve the effective lifespan of the systemic fungicides in controlling GLS, resistance management strategies should be followed. The use of mixtures of unrelated fungicides with different modes of action is a basic component of fungicide resistance management. Rotations of fungicides with different modes of action is an alternative strategy (Delp, 1980 and 1984; Staub and Sozzi, 1984; Georgopoulos, 1986; Wolfe and Barret, 1986; Dekker, 1986; Delp, 1988; Scheinpflug, 1988; Wade, 1988). The availability of a range of fungicides would offer the option of delaying the development of fungicide-resistant strains of *C. zeae-maydis* if deployed wisely. As only two groups of fungicides, the triazoles and benzimidazoles, are currently registered and commercially available in South Africa, other fungicides with different modes of action are needed to control GLS.

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CHAPTER 10

Maize Hybrid Response to Grey Leaf Spot Under Two Tillage Systems In South Africa[16]

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 response of commercial hybrids to the disease was assessed under conventional and stubble tillage systems. The hybrids that were most susceptible to GLS had lowest yields under both tillage practices. Linear regression of relative yield against relative disease severity identified high yielding maize hybrids that were more resistant 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 tillage systems, indicating that the beneficial practice of stubble tillage can be used in areas where GLS is present. Although GLS appeared on plants under reduced-tillage up to three weeks earlier than on the ploughed field under specific environmental conditions, a lower decrease in yield per unit increase in disease was observed under stubble tillage than under conventional tillage.

10.1 INTRODUCTION

Grey leaf spot (GLS) is a foliar disease of maize (*Zea mays L.*) caused by the fungus *Cercospora zeae-maydis* (Tehon and Daniels, 1925) which was first observed near 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

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16 The data from the Cedara site were from co-operative trials conducted by J.M.J. Ward. The chapter has been accepted and will be published as: Ward, J.M.J., Nowell, D.C., Laing, M.D. and Whitwell, M.I. 1997. Maize hybrid response to grey leaf spot under two tillage systems in South Africa. European J. Plant Pathol. (In Press).

quality of maize grown for silage. Nutter[17] (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 USA.

Stubble tillage offers maize farmers and the environment many advantages by reducing soil erosion and water loss, and enabling a lower cost of production. However, increases in the severity and distribution of GLS in the USA has been associated with no-tillage maize (Leonard, 1974; Roane *et al.*, 1974; Hilty *et al.*, 1979; Latterell and Rossi, 1983; 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 (Latterell and Rossi, 1983, Stromberg, 1986; Huff, *et al.*, 1988). However, in South Africa maize has traditionally been grown under a system of monoculture and few farmers practice any form of rotational cropping (Channon and Farina, 1991). Rotations are unlikely, therefore, to be used as a means of control since farmers are reluctant to change cropping practices. Genetic resistance provided 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 GLS-resistant genotypes in South Africa (Gevers *et al.*, 1994, Hohls *et al.*, 1995). However, no commercially available hybrids highly resistant to GLS have so far been released in South Africa, and chemical control methods are being used as an interim solution (Ward and Nowell, 1997).

The purpose of this study was to evaluate and identify high yielding maize hybrids that were resistant 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.

10.2 MATERIALS AND METHODS

Trial data

The trials were conducted at the Cedara Agricultural Development Institute at Cedara, (29°31'S, 30°17'E and an altitude of 1070 m), and at Pannar (Pty) Ltd., near Greytown (29°02'S,

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17 F.W. Nutter, Dept. Plant Pathology, Iowa State University, Ames, Iowa, USA.

30°31'E and an altitude of 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 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, was hand-harvested to estimate yield. At Greytown, plots were two rows, 6.0 m long and 0.9 m apart, 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 heteroscedacity 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 days 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 duration of the epidemic to enable comparisons between epidemics of different durations. The standardised AUDPC was compared to single point model of disease severity 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.

Statistical analysis

Eighty-five hybrids were evaluated at Cedara and Greytown over the three rowing seasons. However, only data from 24 of the hybrids (Table 10.1), common to the three years of study, were used in the analysis of variance.

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 Version 2.2.

The Bestest analysis (Gupta, 1965), further developed by Calitz (1991), and van Aarde (1993 and 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:

$$Y = B_o + B_i X_i + E_i$$

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), X_i 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 and Statgraphics 4.0.

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.

10.3 RESULTS

10.3.1 Disease severity

Disease levels were relatively low in the 1992/93 season due to the prevailing drought, being 3.80 and 32.92% ($\pm$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 (χ^2 = 47.9, $P \leq 0.001$). The same test over the 1991/92 and 1993/94 seasons showed the variances to be homogeneous (χ^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).

Effects of tillage at Cedara

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 10.1 - 10.3).

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, $P \leq 0.001$). There was consistently more disease at Cedara (49.83%) than Greytown (36.85%) (Table 10.2).

Table 10.1: Actual and relative disease and yield of 24 maize hybrids under stubble and conventional tillage systems

Hybrid	Stubble Tillage[1]				Conventional Tillage[2]			
	Disease [3]		Yield [4]		Disease [5]		Yield [6]	
	Actual [7]	Relative [8]	Actual	Relative [9]	Actual	Relative	Actual	Relative
	%	%	kg ha^{-1}	%	%	%	kg ha^{-1}	%
CRN 3584[10]	**24**	**44**	**6970**	**116**	**28**	**113**	**7754**	**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**

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

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

3 Relative yield is calculated by dividing the yield by the trial mean and multiplying by 100.

4 Least susceptible hybrid subset ranked by Bestest analysis have ≤35% disease and most susceptible hybrids have >56% disease.

5 Highest yielding hybrids ranked by Bestest analysis have >6580 kg ha^{-1} and lowest yielding hybrids have ≤6000 kg ha^{-1}.

6 Least susceptible hybrids ranked by Bestest analysis have ≤35% disease and most susceptible hybrids have >47% disease.

7 Highest yielding hybrids/ranked by Bestest analysis have >7600 kg ha^{-1} and lowest yielding hybrids have ≤7030 kg ha^{-1}.

8 Actual disease is percentage leaf-blighting assessed approximately 21 days before physiological maturity.

9 Relative disease is calculated by dividing disease percentage by the trial mean and multiplying by 100.

10 The 9 hybrids used for further analyses are in bold.

10.3.2 Grain yield

Effects of tillage

Table 10.2: 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		
DISEASE SEVERITY (%) [1]			
Tillage	**1991/92**	**1993/94**	**Mean**
Conventional	32.08	67.57	49.83
Stubble	26.42	83.68	55.05
F-test	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-test ($P \leq 0.05$)	*	NS	*
Standard error	3.50	8.90	3.80
CV%	33.4	14.9	11.9
GRAIN YIELD (KG HA^{-1})			
Tillage	**1991/92**	**1993/94**	**Mean**
Conventional	7557	4798	6177
Stubble	8161	4480	6321
F-test ($P \leq 0.05$)	*	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-test ($P \leq 0.001$)	**	**	**
Standard error	95.80	85.10	64.10
CV%	8.0	8.9	8.4

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

Grain yields for 1991/92 and 1993/94 are presented (Table 10.2). 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 ($P \leq 0.05$). 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 10.2). Tests for heterogeneity of variance over the three seasons were homogeneous (χ = 1.378 N.S.). Over all seasons there were no differences between tillage practices.

Table 10.3: Response of relative [a] grey leaf spot severity of maize and relative [a] grain yield representing 24 hybrids under conventional stubble tillage at Cedara and Greytown during two to three seasons

Location	Tillage	% variance accounted for (R^2)	Regression parameters	
			Slope	Intercept
Cedara	Conventional	50.7 **	-0.2082 ** -3.087 ± 0.622**	131.26 ± 6.59
Greytown	Conventional	11.9 *	-0.0662 * -0.0961 ± 0.074 NS	110.45 ± 5.08
Cedara	Stubble	59.0 **[b]	-0.1729 ** -0.2268 ± 0.0388**	122.97 ± 4.28

[a] Values expressed as a percentage of the trial mean
[b] ** highly significant ($P \leq 0.001$)
* significant ($P \leq 0.05$)

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 10.2).

10.3.3 Effect of grey leaf spot on grain yield

Grain yield of 79 hybrids was regressed against disease severity for 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 $P \leq 0.001$), 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 X season interaction, reflecting that the effect of tillage practices on GLS affected yields consistently over seasons in the regression model. There was, however, a significant interaction of location X season, and disease severity X season. The final model including differences in location, tillage season and the interaction of disease and season accounted for 80.3% of variation.

10.3.4 Hybrid response to GLS

Disease severity and yield of 24 maize hybrids are presented on a relative basis (Table 10.1). 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 produced 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 10.1).

Overall, hybrids with low GLS levels had higher grain yields under both conventional and stubble treatments than hybrids with high GLS levels. 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 10.3). 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 (Figures 10.1a and c). Of the susceptible hybrids with more GLS than the trial mean hybrids, RS 5206 yielded as predicted, PAN 6552 yielded higher than predicted, 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 (Figures 10.1a and c).

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 10.1b).

Data of the nine selected hybrids was grouped in less susceptible, intermediate and highly susceptible disease categories. Yield was 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 differences between disease severity and hybrid group (R^2 = 53.3, $P \leq 0.001$) (Figure 10.2). Genstat pair wise test confirmed the presence of these differences ($P \leq 0.05$).

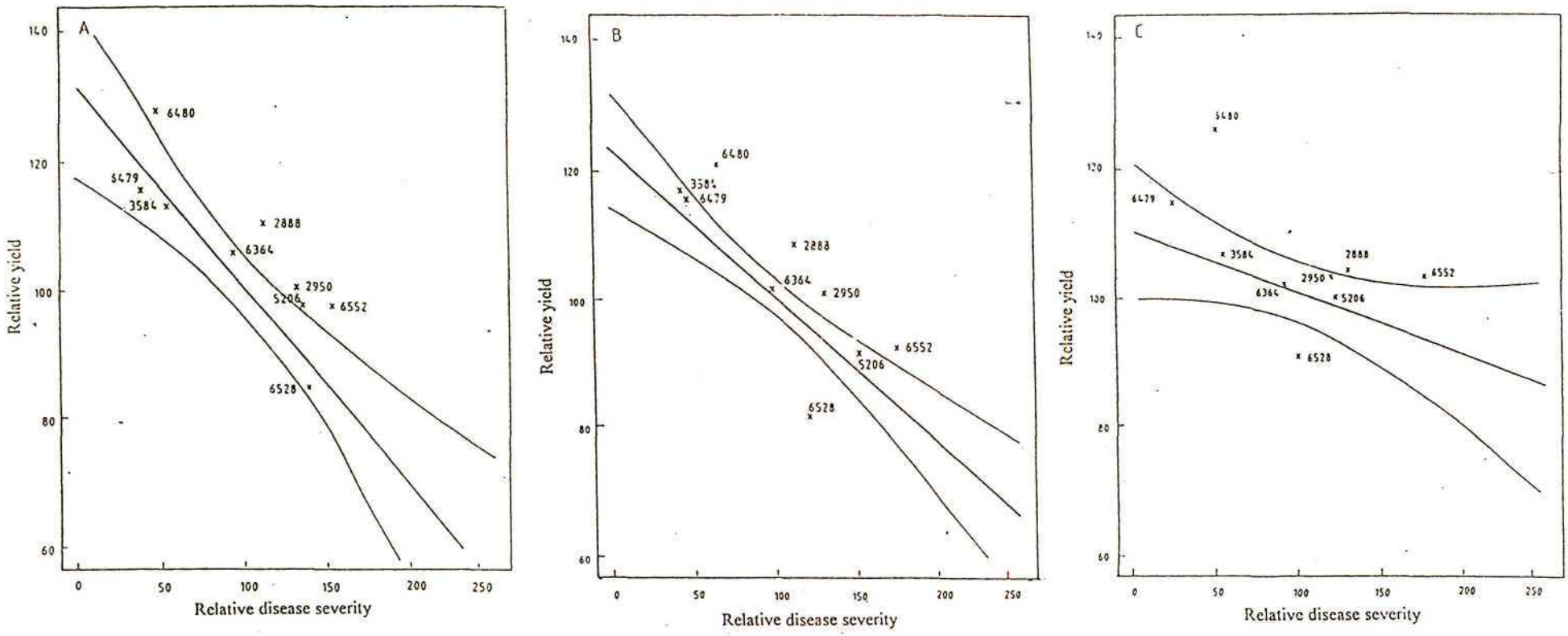

Figure 10.1: Regression analyses of relative grain yield against relative grey leaf spot severity at Cedara and Greytown under two tillage systems: (A) Cedara conventional tillage; (B) Cedara stubble tillage and (C) Greytown conventional tillage. Confidence limits of 95% are shown. The mean of only nine hybrids regressed are shown: PAN 6479, PAN 6480 and CRN 3584 are least susceptible, PAN 6528 and RS 5206 are most susceptible, SNK 2888 and PAN 6552 are tolerant and PAN 6364 and SNK 2950 are intermediate in their reaction to grey leaf spot.

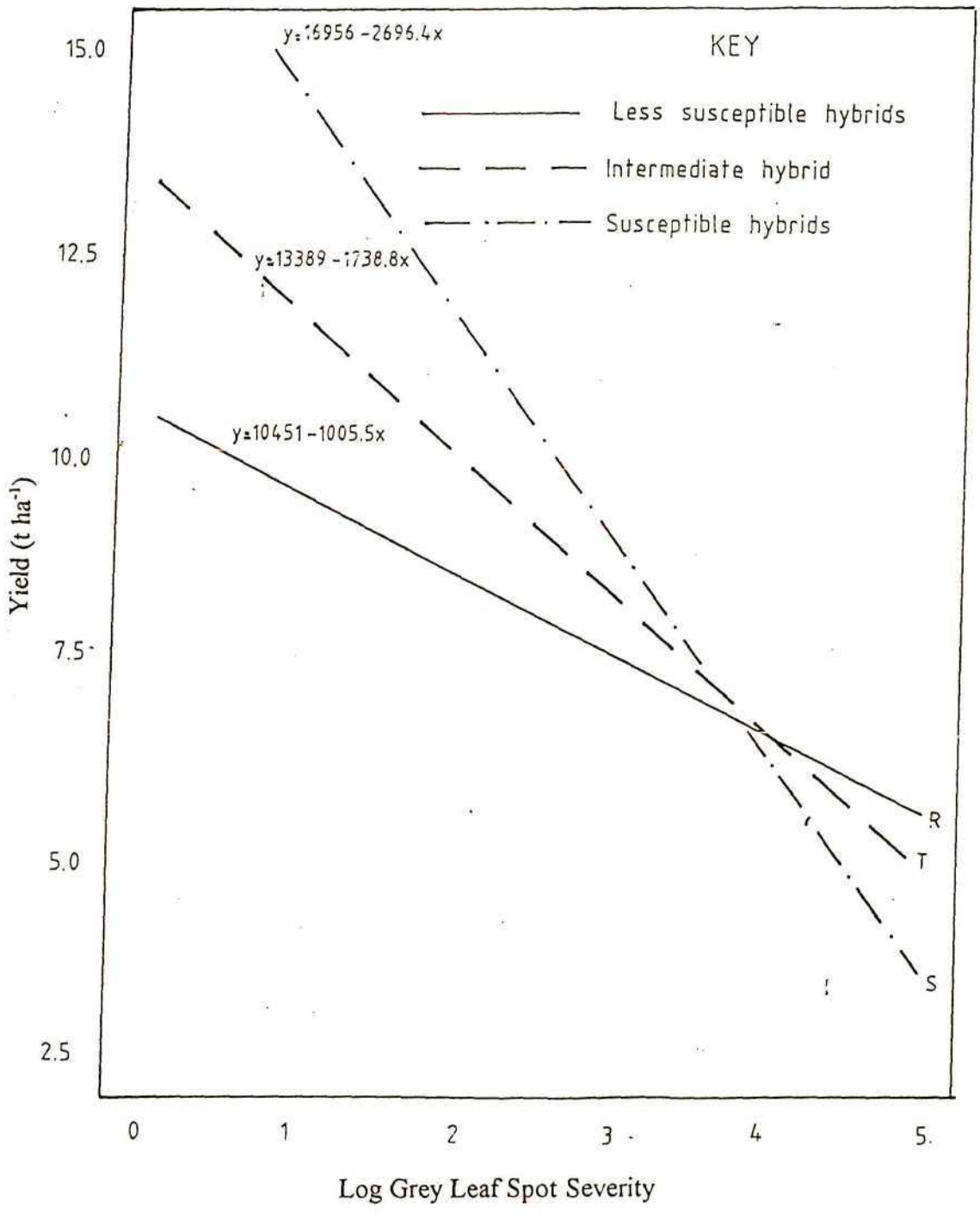

Figure 10.2: Regression analysis of grain yields against log-transformed grey leaf spot disease severity of less-susceptible, intermediate and susceptible grouped of hybrids. The hybrids were evaluated over two seasons across two locations under stubble and conventional tillage systems.

10.3.5 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. The mean number of days to 1% disease for resistant hybrids in 1991/92 was 77 days and for susceptible hybrids was 58 days. In 1992/93, this was 107 days for resistant and 99 days for susceptible hybrids and in 1993/94 this was 79 days for resistant and 76 days for susceptible hybrids (Table 10.4, 10.5 and 10.6).

Less susceptible hybrids had significantly lower disease (AUDPC) in all seasons than susceptible hybrids, except for the drought season of 1992/93, and these lower disease levels were reflected in higher grain yields than susceptible hybrids (Table 10.4, 10.5 and 10.6).

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) ($P \leq 0.001$). There were no differences in yields obtained under stubble or conventional tillage in the three seasons of study.

All correlations that were significant reflected a negative correlation existed between GLS and grain yield, and confirmed that the most susceptible genotypes had the lowest grain yields (Table 10.7).

Table 10.4: Days after planting (DAP) to 1% disease, standardised AUDPC and grain yields for nine maize hybrids under two tillage systems at Cedara during 1991/92

	DAP to 1% disease			AUDPC[2]			Yield (Kg ha^{-1})		
	Tillage[3]								
Hybrid	**Conv**	**Stub**	**Mean**	**Conv**	**Stub**	**Mean**	**Conv**	**Stub**	**Mean**
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 ($P \leq 0.05$)	NS	NS	9	NS	NS	6.5	743	743	525

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

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

[3] Tillage - "Conv" is clean cultivation and "Stub" is stubble tillage.

Table 10.5: Days after planting (DAP) to 1% disease, standardised AUDPC and grain yields for nine maize hybrids under two tillage systems at Cedara during 1992/93

	DAP to 1% disease			AUDPC[2]			Yield (Kg ha^{-1})		
	----------Tillage[3]----------								
Hybrid	Conv	Stub	Mean	Conv	Stub	Mean	Conv	Stub	Mean
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 ($P \leq 0.05$)	NS	NS	4.9	3.4	3.4	2.4	NS	NS	650

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

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

[3] Tillage - "Conv" is clean cultivation and "Stub" is stubble tillage.

Table 10.6: Days after planting (DAP) to 1% disease, standardised AUDPC and grain yields for nine maize hybrids under two tillage systems at Cedara during 1993/94

	DAP to 1% disease			AUDPC[2]			Yield (Kg ha^{-1})		
	----------Tillage[3]----------								
Hybrid	**Conv**	**Stub**	**Mean**	**Conv**	**Stub**	**Mean**	**Conv**	**Stub**	**Mean**
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 ($P \leq 0.05$)	4.3	4.3	3.1	N S	N S	8.2	N S	N S	720

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

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

[3] Tillage - "Conv" is clean cultivation and "Stub" is stubble tillage.

Table 10.7: Phenotypic 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**[(1)]	-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**

[(1)]** Correlation highly significant ($P \leq 0.001$)

* Correlation significant ($P \leq 0.05$)

10.4 DISCUSSION

Grey leaf spot is the most yield limiting disease of maize in KwaZulu-Natal. No commercial hybrids are highly 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 USA 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 in areas, the disease is a problem under favourable weather conditions in both stubble and conventional tillage. In a dry season, the pathogen may infect maize earlier under stubble, but subsequent slow disease development and its effect on yield is offset by improved yield from the beneficial effects of increased soil moisture retention. 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 resistant to GLS will have an advantage, as disease develops in these groups later than hybrids more susceptible to GLS (Table 10.4, 10.5 and

10.6).

Linear regression models used in this study consistently showed that hybrid groups resistant to GLS, with lower GLS levels, had higher grain yields than hybrids more susceptible to GLS. This suggests that less-resistant hybrid groups may have some form of 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). This is illustrated by PAN 6480 which yielded consistently higher than predicted by its GLS score (Figure 10.1). The hybrid groups, susceptible to GLS and with higher than average GLS levels, varied in their predicted yield responses. PAN 6552 had relative yields higher than predicted, indicating it to have some degree of tolerance, but its relatively low yields in the presence of GLS precludes its use in areas where the disease is a problem. 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, had higher than predicted yield responses, indicating this hybrid has tolerance to GLS. The ability of linear regression models to differentiate maize hybrids by relative yields and disease levels into resistant, susceptible and tolerant categories, shows this approach to be 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 10.1), 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 Tukey's 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 resistant hybrids. The earlier appearance of GLS with correspondingly higher levels of disease (AUDPC) in susceptible

hybrids renders this group to greater risk from GLS, as they are subjected to blighting over a longer period than hybrids that are resistant 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 necessitate more spray applications for control than resistant 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 resistant 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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SECTION III - GENERAL DISCUSSION

GENERAL DISCUSSION

For the purposes of this discussion the *Stenocarpella* ear rot and grey leaf spot (GLS) will be discussed separately. Common points will be dealt with under management strategies at the end of the General Discussion.

1 EAR ROT

1.1 Significance of problem

The last ten years have seen changes in the emphasis placed on *Stenocarpella maydis* (Berk.) Sutton ear rot of maize by agronomists and researchers alike in South Africa. The reason for this was the severe ear rot epidemics experienced in South Africa during the late 1980s (Table 4 - General Introduction). During the 1970s and early 1980s, research emphasis was placed on stalk rots as a result of the increased incidence of this disease during the severe droughts in South Africa during this period. The objective was to identify stalk rot resistance and/or management techniques to reduce the incidence and severity of this disease. During this period there was a build-up of *S. maydis* inoculum which causes both stalk and ear rot (Koehler, 1959 and 1960). This ultimately resulted in the ear rot epidemics during the late 1980s when climatic conditions were more suitable for ear rot infection and development in South Africa. This has lead to a change in emphasis placed on *Stenocarpella* ear rot research by both agronomists and researchers in South Africa over the past decade.

The ear rot epidemic started in 1985/86 in KwaZulu-Natal province before becoming a significant problem and spreading throughout the country. The greatest effect of this epidemic was in the eastern maize production regions of the country where yellow-grained maize is primarily produced. The disease had a marked impact on the grading of maize on delivery to the storage silos from the 1986/87 season to the end of the 1988/89 season (Table 4 - General Introduction). White-grained maize is primarily produced in the western maize production regions of South Africa where the climate is not normally conducive to ear rot infection and development. Consequently the ear rot epidemic lagged behind in these areas until the inoculum levels and climatic conditions were conducive for ear rot. The ear rot epidemics did not materialise again once the drier seasons of the late 1980s and early 1990s arrived.

The incidence of ear rots was particularly severe in Mpumalanga and KwaZulu-Natal. Of the maize delivered to the silos, over 90 percentage of the rotten kernels yielded *Stenocarpella* (Visser[18], pers. comm.). Discussions with maize farmers leave the impression that although the majority of "Diplodia" infections are evident, the severity of disease is not apparent until the field is harvested. This "hidden" ear rot was often not taken into account when estimates were made. This is supported by the estimates of ear rot undertaken by researchers in Greytown, KwaZulu-Natal (Chapter 3) where ear rot severity was consistently underestimated. Many farmers had to mix their diseased and healthy maize before delivery to the silos so that a reasonable quality and price could be obtained.

1.2 Distribution of *Stenocarpella* species

Stenocarpella macrospora (Earle) Sutton is limited in distribution to southern / central Mpumalanga and KwaZulu-Natal, which are the higher rainfall regions of South Africa (Marasas and van der Westhuizen, 1979; Rheeder, 1988; Flett, 1990; Rheeder *et al.*, 1990; Rheeder and Marasas, 1992 and 1994). In 1987 in the Midlands of the KwaZulu-Natal province, a seed production field was determined to have approximately 40% leaf area loss due to *S. macrospora*. Approximately 25% of the ears were rotten with ≥95% of the infection caused by *S. macrospora*. During the mid- to late-1980s, a number of fields with ≥25% leaf area loss caused by *S. macrospora* were observed in this region. These infections occurred on a variety of hybrids with varying degrees of resistance.

S. maydis was found to occur throughout the maize productions regions of South Africa and this pathogen was usually the predominant species, particularly in the Highveld and western production regions (Rheeder, 1988; Flett, 1990; Rheeder *et al.*, 1990; Rheeder and Marasas, 1992 and 1994). This distribution is expected, based on the information provided by Latterell and Rossi (1983a) for its climatic requirements in other parts of the world.

1.3 Quantification of yield loss and quality loss

Although there have been large losses due to ear rot in the recent past, no information is

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available on the economic losses or the exact quality problems associated with these losses. Without this type of information, it is difficult to justify extensive and costly research programmes. This information should have been readily available from various bodies controlling the maize industry in the past; e.g., Cronje *et al.* (1994) who discussed maize quality concerns from a marketing perspective. Not only is poor grade maize more difficult to sell, but grain protein content appears to be reduced. However, less nitrogen was applied to the maize during this period to try and reduce production costs (Cronje *et al.*, 1994), confounding the interaction of ear rot and grain protein content. Exact effects of ear rot on various grain quality aspects remain unknown and need to be established.

1.4 Rotation

Crop rotation is widely practised in countries with developed economic and agricultural infrastructures; i.e., first world countries. However, South Africa does not fall into this category and crop rotation is not practised widely (Channon and Farina, 1991). It is clear from many papers that crop rotation not only decreases ear rot/disease, but can increase yield and hold down costs of fertilizer and herbicides in maize (Koehler, 1959; Wilcoxson and Covey, 1963; Williams and Schnitterhenner, 1963; Kerr, 1965; Shipton, 1977; Palti, 1981; Sumner *et al.*, 1981; Sumner and Bell, 1986; Jamil and Nicholson, 1987; Windels *et al.*, 1988; Reagan, 1989). Reagan (1989) suggested not only rotating crops but also hybrids, tillage practices and pesticides. This would allow farmers to obtain maximum benefits from all variable factors. The benefits of a fallow period in a rotation system were apparent and a three-year ecofallow system introduced in Nebraska, USA, had become popular (Sumner *et al*, 1981).

Crop rotation is the single most important factor that could be used to reduce the incidence of ear rot in maize in South Africa. Unfortunately, there is a lack of literature on the effects of rotation on *S. maydis* ear rot, particularly under local conditions. Early unpublished research in South Africa in 1986/87 (Table 5), showed that crop rotation benefits with regards both grain yield and ear rot severity could be large. This trial was planted late in the season and the trends obtained can be expected to be the minimum for earlier planted maize, as ear rot incidence and severity is usually greater in early planted maize.

Table 5: Effect of crop rotation on maize grain yield and grain disease at Greytown, KwaZulu-Natal (Farwell[19], unpublished)

Type *	Rotation Crop	Yield(T/Ha)		% Yield Benefit	% Dis Grain		% Ear Rot Benefit
		Monoculture	Rotation		Monoculture	Rotation	
S	Wheat, peas and beans	6.49	7.43	15	<6	<6	0
S	Virgin soil	5.22	6.93	33	27	19	30
S	Silage	6.94	7.69	11	38	20	47
I	3 Seasons vegetables	1.20	2.46	105	85	31	64

* S = single cross I = inbred

A rotation trial conducted by the Oos Transvaal Kooperasie (O.T.K.), near Bethal in Mpumalanga (Table 6), clearly showed an ear rot reduction after crop rotation. It is interesting to note that a season of fallow resulted in the greatest reduction in disease, suggesting rapid reduction in inoculum viability and supporting Sumner *et al.* (1981). The grading figures in Table 6 were those received by the farmer at the grain silo on delivery of his crop. With this kind of reduction in maize ear rot due to crop rotation, it should become a common practice in maize production in South Africa.

Table 6: Effect of rotation on the incidence of ear rots in the Mpumalanga province during the 1986/87 season (Koster[20], unpublished)

Rotation System	% *S. maydis* Diseased Ears	% Ear Rot Benefit	Grading of commercial farmer
Maize / maize	9.8	0	Third
Maize / wheat	4.6	53.1	First
Maize / beans	4.1	58.2	First
Maize / fallow	2.7	72.4	First

Klemme *et al.* (1995) showed that the gross margin per hectare for maize monoculture was lower than either maize-soybean or maize-soybean-wheat rotations at two sites in the USA. Not only can disease affect crop grain yield but it can also significantly influence the quality of the product. Where this occurs the value of the grain would be reduced further. There is no information available in South Africa on the economic value of crop rotation in maize production

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systems.

For a pathogen such as *S. macrospora* there would be the added advantage of a reduction in the leaf blight phase, as well as a reduction and/or elimination of primary inoculum. It is important to consider rotation in conjunction with tillage practices, as this would influence the duration of the rotation cycle, type of crop/s used in rotation and equipment needed. Ideally, rotation should be practised with the no-till tillage system, as this allows for maximum utilisation of moisture and keeps production costs to a minimum. However, the problem is that with maize the plant debris is left on the soil surface, so the duration between the same crops would have to be longer if compared to a plough-tillage system as the colonised maize debris would need longer to breakdown with zero-till. A rotation period of three maize growing seasons is usually considered sufficient.

A current development in parts of South Africa is the installation of irrigation systems and the planting of winter crops which could reduce the period between maize crops. An additional factor that would influence the rotation period is that of animals grazing the stalks after the crop is harvested. Animals are allowed to graze on the colonised maize debris after the crop is harvested and before the fields are tilled. This reduces the need for alternate feed, and the costs associated with this practice, and reduces the amount of colonised maize debris on the soil surface.

1.5 Tillage

When South Africa entered a cycle of very dry years in the early-1980s, the emphasis in the extension service turned towards conservation of soil moisture, yield stability and a reduction of production costs. The emphasis on soil moisture conservation through conservation tillage, resulted in crop debris being left on the soil surface to reduce erosion and moisture evaporation. When the environment became more moist, particularly when two moist seasons occurred in a row, then a series of *S. maydis* ear rot epidemics arose as a result of the increased inoculum on the debris in the field.

The basic plant pathological practice of sanitation (Vanderplank, 1963 and 1975; Zadoks and Schein, 1979) is very important in maize ear rot (Koehler, 1959; Kerr, 1965; Palti, 1981), yet in 1986 (at the start of the ear rot epidemic in South Africa), there was no literature published on the effect of tillage practices on ear rot in South Africa.

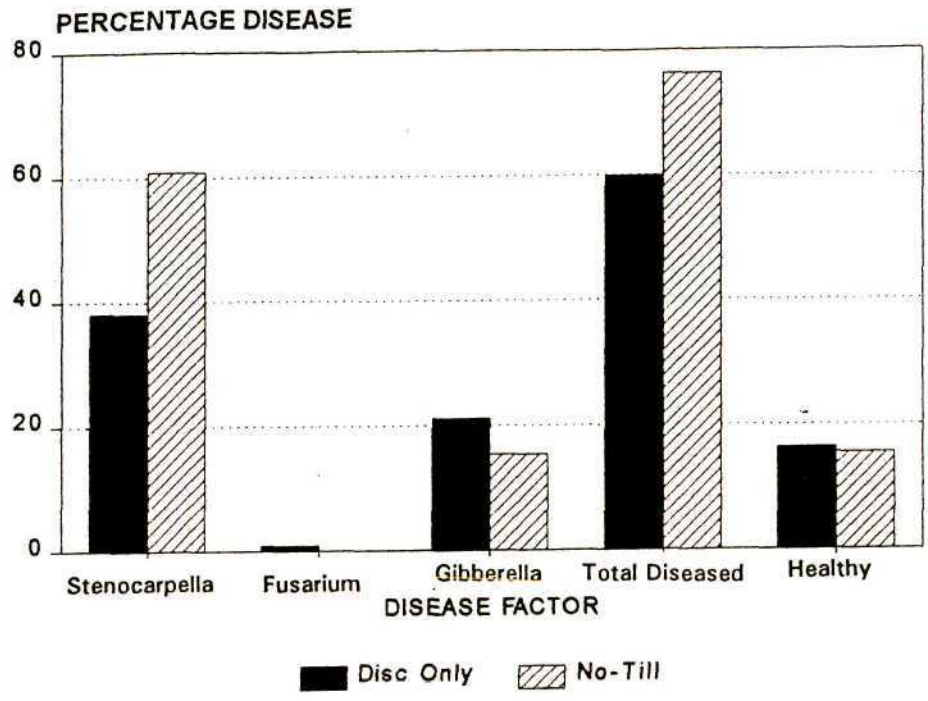

Figure 1: The effect of two tillage systems on the incidence of ear rot in the Greytown area in 1985/86 (Nowell, unpublished).

Table 7: The effect of tillage practices on the incidence of *Stenocarpella* spp. (Koster, unpublished)

Tillage Practice	Percentage Colonised Ears			
	1985/86		1986/87	
	% DisGr	Rel. %	% DisGr	Rel. %
No-Till	4.0	173.9	29.3	279.0
Minimum Till	3.5	152.2	23.0	219.0
Rip and Disc	1.7	73.9	12.7	121.0
Disc X 2	1.5	65.2	12.3	117.1
Summer Plough	1.5	65.2	11.5	109.5
Winter Plough	2.3	100.0	10.5	100.0

% DisGr - actual percentage diseased rotten ears.
Rel. % - percentage disease relative to the Winter Plough treatment which is taken as the control as this is the most common period of ploughing.

An initial survey undertaken near Greytown in KwaZulu-Natal in 1986, showed that no-till increased *S. maydis* ear rot incidence in a seed production field when compared to chisel-tillage practices (Figure 1). Unfortunately this field did not have an area that had been ploughed.

This trend was supported by Koster (unpublished) who showed an increase in *Stenocarpella* ear rot with an increase in maize debris on the soil surface (Table 7). The effects of tillage were more pronounced in the 1986/87 season when climatic conditions were ideal for ear rot development and ear rot became a regional problem. No-till increased ear rot regardless of the prevailing environmental conditions.

Table 8: Selected data from the Grain Crops Research Institute's tillage trials conducted in KwaZulu-Natal during the 1986/87 season (Mallet and Berry[21], unpublished)

Site	Cedara	Dundee	Winterton	Geluksburg
Hybrid	PAN 6549	PAN 394	PAN 6549	RS 5206
Planting Date	16/10/86	04/11/86	11/1186	10/11/86
Tillage Treatments				
Disc, mouldboard, disc				
Yield (kg/ha)	7052	4895	5853	2138
% Diseased ears	17.7	18.7	9.3	32.5
Disc, chisel plough, disc				
Yield (kg/ha)	7051	5362	6640	2002
% Diseased ears	15.1	21.8	9.5	37.4
Chisel, chisel				
Yield (kg/ha)	7283	5921	6747	5202
% Diseased ears	21.8	24.8	18.3	28.5
No-till				
Yield (kg/ha)	7265	5757	6451	3947
% Diseased ears	20.1	31.5	30.8	56.1
Significance				
Grain yield	NS	NS	NS	*
Diseased grain	NS	**	**	**

* - significant at the 5% level.
** - significant at the 1% level.

Trial results released by Mallet and Berry (unpublished) in 1987 again showed the high increase in ear rot associated with the zero-tillage system at various locations in KwaZulu-Natal (Table 8). The trial at Cedara did not give rise to any differences in ear rot incidence but the other three locations all showed differences between treatments. The hybrids PAN 6549, PAN 394, PAN 6549 and RS 5206 were planted at Cedara, Dundee, Winterton and Geluksburg,

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respectively, but PAN 394 is the only hybrid that was considered susceptible to ear rot in commercial maize production. There was significant mid-season stress at the Winterton and Geluksburg locations, which predisposed these hybrids to infections leading to ear rot with the late rains. It is revealing to see the increases in the percentage rotten ears of 68.4%, 231.4% and 72.6%, at Dundee, Winterton and Geluksburg, respectively, under zero-tillage when compared to the mouldboard plough treatment. Whether the trend at Cedara was a single season effect or representative of previous seasons was of concern as this was one of the sites where initial research in tillage methods and maize was conducted. This may be the reason why ear rot had not been observed to increase with reduced tillage earlier in South Africa. The Cedara site has also shown to have the highest incidence of *S. macrospora* ear rot (Rheeder, 1988; Flett, 1990; Rheeder *et al.*, 1990; Rheeder and Marasas, 1992 and 1994).

The benefits in moisture conservation under conservation tillage methods are obvious from these yield data. It is for this reason that farmers must be encouraged to practice conservation tillage but should be made aware of the potential disease problems, particularly ear rot, associated with this practice and make alternate plans to reduce the risk of ear rot.

Many farmers have expressed the view that if they plough their fields they will reduce the incidence of ear rot. The effectiveness of ploughing is largely dependant on how effectively the colonised maize debris is buried. Often ploughs are not adjusted correctly and a significant portion of the debris is either not buried or only half-buried. It is important to bury as much debris as is practically possible, otherwise it will readily provide inoculum for infection the following season (Lipps, 1983; Flett, 1990), and there is an increased risk of moisture stress due to the reduced conservation of moisture with ploughing. This in turn can predispose the maize to increased levels of ear rot.

1.6 Burning

Burning of *Stenocarpella*-infected maize debris should only be considered as a last resort due to the negative impact this practice could have on the organic matter content of the soil and the potential for soil erosion during rain. It is unlikely that a completely clean burn can be obtained; i.e., 100% of the maize debris is destroyed. This means that ear rot inoculum would not be completely eliminated, especially in the colonised root and basal stem debris which is still in the soil. Burning may therefore substantially reduce the amount of inoculum, but will not

eliminate it.

1.7 Silage

Table 5 shows that the removal of plant material for silage purposes can reduce the incidence of ear rot and increase grain yield in the subsequent season. This is in effect of the removal of most of the inoculum, which reduces the likelihood of ear rot the following season. Although removal of maize for silage is unlikely to be practised over large areas, the practice will be beneficial as one of the control measures in overall integrated disease management. The areas used to produce silage on a given farm can be rotated to spread the benefit of this practice over the farm as a whole.

1.8 Seed-borne aspects

The influence of *Stenocarpella* seedborne inoculum is likely to be insignificant in terms of the epidemiology of the root, stalk and ear rot complex under South African conditions. There are so many potential sources of inoculum, particularly *Stenocarpella*-infected maize plant debris, that seed-borne inoculum is not significant. Research in the USA suggests that seed-borne pathogens of maize under USA conditions do not increase root and stalk rot (Ooka and Kommendahl, 1977; El-Meleigi *et al.*, 1983). No research has been undertaken on *Stenocarpella* ear rot of maize and the importance of seed-borne inoculum in the disease life cycle. It is likely to have even less effect than on root and stalk rots. This could be a very interesting but extremely difficult study to undertake.

Little information is available of the effect of *Stenocarpella* spp. on germination, seedling vigour, seedling blight and systemic infection. Seed companies remove *Stenocarpella*-infected seed because of the pathogen's effect on germination but little detailed information is available on this effect under local conditions. This is also true for seedling vigour and damping-off. Ooka and Kommendahl (1977) and El-Meleigi *et al.* (1983) suggested that seed-borne inoculum has no effect in increasing root or stalk rot. The possibility of *S. maydis* infecting stalks from colonised seed has not been investigated under local conditions. This may be possible in South Africa due to relatively high levels of moisture stress experienced that may predispose the plants to ear rot infection. This infection process could be complex and would need to be investigated under a number of different environmental conditions.

Chemical control has been investigated overseas and found to be effective in increasing germination and seedling emergence, and reducing seedling blight caused by *S. maydis* (Hoppe, 1943; Crosier, 1946; Kruger, 1968). There are a wide variety of fungicides available for the seed treatment of maize but little is known about their effect on *S. maydis*-infected seed (Jeffs, 1986). Even less is known about the effectiveness of fungicides on seed-borne *S. maydis* infection under South African conditions. Captan is widely used as a seed treatment for maize in South Africa but little research has been done to determine its effectiveness, especially against *S. maydis*-infected seed. Due to the climatic conditions in South Africa, seedling diseases are likely to be less important than in regions with more moist environments. However, seed treatments are employed, and are most effective, for the exceptional conditions rather than the routine. Local research should be undertaken to determine efficacy and cost effectiveness of the various fungicide products available.

1.9 Planting Date

The effect of planting date on *Stenocarpella* spp. is marked, especially in the eastern part of the country. Research in the KwaZulu-Natal Midlands has shown that maize planted after 15 November have a substantially reduced risk of ear rot. The earlier the maize is planted, the greater the risk of *Stenocarpella* ear rot. However, the later maize is planted in this region, the less heat units and rainfall are received during active growth and the lower the grain yield potential is. A compromise would be to plant between 5 and 20 November in order to balance the risks (Farwell, unpublished; Nowell, unpublished). This effect will vary between locations depending upon the seasonal rainfall distribution. It is important to reduce the risk of ear rot by reducing the likelihood of rainfall during grainfill and grain maturity, in order to reduce favourable conditions for the infection and subsequent spread of the ear rot fungi. A balance is necessary between potential grain yield and ear rot risk.

1.10 Infection

Flett and Wehner (1989) found that *S. maydis* conidia could be airborne. The spore type is a slime spore and they are not usually airborne. Slime spores are essentially splash dispersed, which means dispersal cannot take place over more than a few metres. The fact that *S. maydis* conidia can be airborne under certain environmental conditions, and remain viable, means that dispersal is not limited to an area immediately around the inoculum source. More detail is

needed on the distances the conidia can travel and the effect the environment has on conidial dispersal and viability.

Most researchers agree that infection takes place at the base of the ear or through the shank region of the ear (Durrell, 1923; Clayton, 1927; Shurtleff, 1980; Palm and Calvert, 1981; Achar and Rabikoosun, 1995). However, the optimum time for infection varies considerably. Early studies suggested that maximum infection takes place between 0 - 2 weeks after anthesis but inoculation studies have shown infection can takes place up to 6 weeks after anthesis (Koehler, 1959; Kerr, 1965; Villena, 1969; Ullstrup, 1970; Chambers, 1986). Warren and Onken (1981) and Nowell (1992) found that inoculation with a conidial suspension or dry, milled ear rot inoculum was 10 - 14 days before anthesis resulted an ear rot epidemic. These results suggest that environmental conditions at the time of infection are most important and may interact with the method of introducing the pathogen into or onto the plant. The afore mentioned studies suggest that *Stenocarpella* spp. can infect maize ears from anthesis to at least 8 weeks after anthesis, provided environmental conditions are suitable during the infection process.

Eddins (1930) suggested that infection took place through the tip of the ear. Koehler (1959) mentioned that *S. maydis* tip infection had been observed in the field but was infrequent. This form of infection has been observed on a number of occasions in KwaZulu-Natal and is usually associated with moist conditions during the final period of grain fill. Ear rot severity with tip infection or colonisation is usually not as high as when the ear is infected from the base. This suggests infection taking place late resulting in the pathogen having less time or less favourable conditions to develop. Tip infection is invariably caused by *S. maydis* in KwaZulu-Natal.

An area that needs further investigation is to understand the conditions required before *Stenocarpella* spp. will move into the ear from the stalk and cause ear rot. It is likely that this is influenced largely by cultivar resistance and the environment. McLennan (1991) could not show that *Stenocarpella* spp. moved from the stalk below the ear into the ear, but was able to increase ear rot by inoculating the internode below the ear shank. In the 1987/88 season, Nowell (unpublished) found that in maize stalks naturally infected by *S. macrospora* below the ear, the fungus moved through the stalk below the ear and then colonised the ear during the 1987/88 season. During this season there was moisture stress at flowering which probably enhanced the spread of the fungus.

1.11 Symptoms

Where both *Stenocarpella* spp. are prevalent, it is not possible to tell the species apart on colonised ears without examining the conidia under a microscope. If a field of maize is regularly monitored over the whole growing season, then a better indication of the species involved can be obtained. The first indication that *S. macrospora* is involved will be either leaves infected with the fungus (resulting in a blight), or the appearance of relatively large lesions on the leaf sheath which engulfs the whole sheath in 2 - 3 days. This results in the leaf wilting within 1 - 2 days, due to the inability of water to move into the leaf (Nowell, unpublished). This type of infection is frequently seen in fields that had a high incidence of leaf blight caused by *S. macrospora*. *S. maydis* apparently does not attack the leaf sheath readily (Chambers[22], pers. comm.). *S. macrospora* sporulates readily on these lesions and the progression of the fungus up the plant (from the site of infection) is rapid and may spread through four internodes within ten days. The *Stenocarpella* infection seldom moves down a plant from the site of infection, especially if the site of infection is above the ear (Nowell, unpublished). *S. maydis* is not known to move rapidly through the plant (Chambers, pers. comm.). This was similar to the finding of Latterell and Rossi (1983a) who found *S. macrospora* to be more aggressive than *S. maydis*. They suggested a possible reason for this rapid movement of *S. macrospora* through the plant is that *S. macrospora* is able to grow through the vessel elements within the xylem more readily than *S. maydis*.

A problem that arose in the late 1980's was the effect of seed-borne fungi (primarily *Stenocarpella* spp.) on the germination of the seed (Chambers, pers. comm.). Apparently the problem arose as a result of high levels of infection and colonisation during seed production, especially with hybrids susceptible to *Stenocarpella* ear rot. However, it would be thought that good quality controls measures would be sufficient to reduce this problem to a manageable level. A problem can arise when the severity of ear rot increases when the environmental conditions are favourable for the fungus to grow on the ear when the grain moisture is below 28%. "Hidden Diplodia" is late infection and colonisation of the grain by *Stenocarpella* spp. which move up primarily from the shank, cob and the stalk into the grain (Koehler, 1959). As a result of the low kernel moisture during this stage of growth, infection is limited to the more moist

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area of the kernel, i.e., the embryos. This type of colonisation results in visually healthy ears with a high percentage of rotten grain when the ear is shelled. Disease of this nature can easily result in a seed lot having unacceptably high levels of rotted kernels which could reduce germination and seedling vigour or result in seedling blight. The only solution is to break each ear in half (by hand) when cleaning the crop and check for rotten kernels. Certain genetic backgrounds appear to be more susceptible to this type of infection.

1.12 Stress

Dodd (1980a and 1980b) summarised this work with his photosynthetic stress-translocation balance (PS-TB) concept of predisposition of maize to stalk rots. Any form of stress usually results in significantly more stalk rots which he showed was as a result of reduced carbohydrates in the stalk, allowing fungi to infect and/or spread rapidly. This effect occurred regardless of the causal fungus. It has been shown that up to 20 percentage of the grain weight originates from the stalk carbohydrates under normal environmental conditions and this proportion can increase rapidly under stress (Jurgens *et al.*, 1978), resulting in reduced metabolic defence mechanisms and the initiation of senescence which is apparently ideal for fungal proliferation. According to Dodd (1980a and 1980b), this holds for both the roots and lower basal portion of the stalk, with the other plant parts being less affected. According to Schneider and Pendery (1983) the growth stage at which the crop is stressed is important. Their research suggested that mild water stress pre-anthesis resulted in the largest increase in stalk rots. However, this may vary depending on the severity of the stress. It is interesting to note a paper on calorific energy distribution within a maize plant and the redistribution of this energy after pollination (Girardin, 1985) which clarifies the partitioning of carbohydrates in the maize plant, before and after pollination. Field experience in South Africa has shown the same types of moisture stress predispose maize to *S. maydis* ear rot should environmental conditions be favourable for infection during ear development and grain fill.

The exact mechanisms involved are not clearly understood. Pappelis *et al.* (1973) showed that ear rot was related to the number of senescent cells in the cob tissue during grain fill. Pre-anthesis stress would reduce the plant's capacity to produce sufficient carbohydrate to completely satisfy the sink (ear) after pollination has taken place. All carbohydrate would go to the grain leaving little to satisfy the needs of the expanding cob. This would increase the number of senescing cob cells and increase the likelihood of *S. maydis* ear colonisation. Essentially the

difference is that under ideal environmental conditions the amount of surplus or free carbohydrates would be high. However, in plants that have experienced pre-anthesis stress, the amount of surplus or free carbohydrate would be low or negligible and the number of senescent cells would increase. Early and significant colonisation of the cob tissue would result in the rapid colonisation of the ear as a whole. *S. maydis* ear rot is essentially a "high sugar" disease, whereas *S. maydis* stalk rot is a "low sugar disease" as defined by Vanderplank (1984).

1.13 Plant density

Although no detailed information could be found in the literature regarding plant density and *S. maydis* ear rot, it has been accepted in the maize industry that increases in plant densities result in an increase in the incidence of ear rot (Koehler, 1959). Results from experiments near Greytown (Chapter 5) show that a linear relationship does not occur under local conditions. One reason for this is that plant densities used by farmers in southern Africa are different to those used in the first world countries. This is essentially due to the variable climate under which maize is produced locally. Many of the regions in South Africa where maize is produced would in most other countries be considered completely unsuitable for maize production due to their low and variable rainfall. For this reason, local maize breeders have developed highly prolific hybrids that can be planted at low plant densities (as low as 9 000 plants ha^{-1}) that will produce many ears per plant should the climatic conditions be suitable for maize production. In this way the effect of low plant density is compensated for under favourable climatic conditions. The same hybrids must also be able to produce high and stable yields at plant densities over 50 000 plants ha^{-1}. Plant densities in the USA are usually well in excess of 45 000 plants ha^{-1}. When *S. maydis* ear rot is examined in plant densities of 50 000 plants ha^{-1} and higher, there is a definite increase in *S. maydis* ear rot with increased plant density. This supports information from the USA. At plant densities lower than 50 000 plants ha^{-1}, there are two distinct hybrid response patterns. In the one group (usually the more *S. maydis* ear rot resistant group), *S. maydis* ear rot incidence and severity increases with increased plant density. The second group (usually the more *S. maydis* ear rot susceptible group), *S. maydis* ear rot incidence and severity is highest at 35 000 - 40 000 plants ha^{-1}, gets significantly less ear rot around 50 000 plants ha^{-1} and then ear rot generally increases with increased plant density.

This research showed that this pattern is most obvious when conditions are ideal for *S. maydis* ear rot infection and development. This may be the main reason why these response patterns

have not been noted under local conditions before. Initially it was thought that prolificacy may have been responsible or involved in these response patterns, as the hybrids usually become prolific in plant densities less than 50 000 plants ha^{-1}. This theory would have tied in with Dodd's sink/source theory (Dodd, 1980a and 1980b). However, prolificacy was not correlated with ear rot response. In fact, ear rot severity showed very little correlation with any of the factors measured (grain yield, prolificacy, grain moisture and the grain shelling percentage).

The effect of plant density on ear rot needs to be investigated further as the implications for the maize farmers and maize seed producers are large. Most yellow-grained maize produced in the eastern part of the country is produced at plant densities of 35000 - 45000 plants ha^{-1}. A high proportion of the seed maize is produced at these plant densities as well. Trials should include ear rot susceptible and resistant hybrids that are prolific and others that produce single ears only. A wide range of agronomic factors need to be measured. Trials need to be undertaken at plant densities ranging from 15 000 - 90 000 plants ha^{-1} and in at three or more geographically distinct locations (KwaZulu-Natal, Mpumalanga and North West provinces) over two or more growing seasons. In the study undertaken (Chapter 5), the agronomic factors were examined individually in relation to plant density and ear rot response. Future studies should use multiple regression analysis as the relationships involved in these response patterns are likely to be complex. Climatic factors should also be closely monitored during these trials.

Although the trial was relatively small in size, plant density experiments are inherently difficult in practice.

A second option would be to undertake these trials using a small diallel (5 inbreds crossed in all possible combinations) to study the inheritance of *S. maydis* ear rot resistance at different plant densities, instead of known hybrids. Inbreds could be selected to give a range of ear rot responses and prolificacy.

1.14 Irrigation

The effects of irrigation are very important to seed maize producers in South Africa, as a significant proportion of maize seed is produced on farms that have irrigation. This ensures the seed industry can obtain a seed crop even under harsh and variable weather conditions. El-Meleigi *et al.* (1983) showed that irrigation could substantially reduce the amount of stalk rot

by reducing moisture drought stress on the plant. This is generally considered to be one of the benefits of irrigation but no one has apparently looked at the possibility of an increase in ear rot due to irrigation. From data collected in the 1986/87 season in the Greytown area, it was shown that the incidence in *Stenocarpella* ear rot could double under centre pivot irrigation. Late plantings, when the risk of ear rot is lowest, that were irrigated also increased in the incidence of *Stenocarpella* ear rot beyond what is normally expected (Nowell, unpublished). This means that centre pivot irrigation should not be used in seed production fields unless absolutely necessary, or applied in such a way as not to increase the leaf wetness period or increase fungal spore production. It is likely that other forms of irrigation (i.e. flood and drip irrigation) may not have as marked an effect in increasing ear rots, as the plant surface is not moistened and there is no splashing of the *S. maydis* conidia.

1.15 Herbicides

There are some reports that certain herbicides can increase the prevalence of ear rot fungi (Nelson[23], pers. comm.). There were also indications that some of the herbicides used when planting maize may also increase stalk and ear rots in the Greytown area (Nowell, unpublished). The trends observed were small but need to be investigated further. Such trials would have to be well designed and include a wide range of herbicides, and herbicide mixes, that are used commercially. The trials would have to cover a wide range of environmental conditions to cover any eventuality, including soil type and climate.

1.16 Hybrid response

Probably the single most important environmental factor influencing *S. maydis* ear rot in South Africa, is that of moisture (drought) stress before or near anthesis. The majority of the maize producing areas in South Africa are classified as marginal crop farming lands from the point of view of rainfall, and usually experience at least one period of drought stress during any given growing season (Cronje[24], pers. comm.). There are many areas, especially in the drier western maize producing regions of South Africa, which during the late 1980's and early 1990's experienced more crop failures than successes due solely to drought. In the sporadic wet periods

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in some regions during the late-1980s, maize that had been subjected to stress and then moist conditions after flowering, was predisposed to infection by *S. maydis* and subsequent development of ear rot (Berry and Mallett, 1992; Koster, pers. comm.; Nowell, unpublished). The alarming observation was that this was regardless of the inherent levels of resistance to *S. maydis* in the hybrids. This means that resistance to *S. maydis* cannot be completely effective in eliminating ear rot because periods of moisture stress occur before anthesis could negate any ear rot resistance effect.

The stress effect has resulted in maize researchers in South Africa being highly cautious when classifying resistance to *S. maydis* ear rot. The South African Agricultural Research Council has opted to classify hybrid response to *S. maydis* ear rot ranging from highly susceptible to least susceptible. They are of the option that resistance to *S. maydis* ear rot cannot be used as all resistance could be negated by the stress effect. This form of categorisation will not give rise to the false impression that certain hybrids are ear rot resistant and will not be severely affected by *S. maydis* ear rot (Flett[25], pers. comm.). The problem with this philosophy is that farmers, and the maize industry in general, are not necessarily made fully aware of the large differences in *S. maydis* ear rot response between current commercial hybrids. There are a number of hybrids that can be classified as being resistant to *S. maydis* ear rot. Education is needed to deal with the problem of stress predisposing maize to ear rot infection.

The non-linear *S. maydis* ear rot response model proposed by Flett and McLaren (1994) largely explains the variation in hybrid response to ear rot. This has led to a far greater understanding of the commercial hybrid response to *S. maydis* ear rot and allowed for sound recommendations to the farmers in high risk ear rot areas. However, there are still some hybrids that do not seem to completely fit into this model. A further refinement could be to investigate location or geographic effects, which may explain some of the variation still present in some hybrids. An additional problem is that of availability of the software to other researchers in the maize industry. Currently the software is complex and not user-friendly. If such a programme was available to others it could be used to further ear rot screening and breeding at other organisations. It is also likely that this model could be used for other maize diseases as well; e.g., GLS.

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Currently *S. maydis* ear rot screening, and non-linear regression analysis, is only conducted on the commercial hybrid series (49 hybrids annum^{-1}). Ideally for ear rot screening this should be extended to include the pre-commercial hybrid series (Phase I) so that when hybrids are released to the farmers on a commercial scale the ear rot information is already available. Currently there is a delay of at least one season from the time a hybrid is commercialised until the farmer can get accurate and reliable *S. maydis* ear rot response information. Instead of being screened at 3 - 4 locations a season, each hybrid series should be screened at two locations only. This preliminary information could then be expanded in subsequent seasons. This approach would reduce the risk of crop failure due to *S. maydis* ear rot in the first year or two of commercialisation.

1.17 Resistance / Genetics

In maize breeding programmes, the emphasis turned towards hybrid adaptability and yield stability in the early-1980s, to reduce the effects of the variable weather. The emphasis on disease resistance was reduced due to the lack of natural disease pressure (in the RSA, maize breeders had relied on natural infection to screen their germplasm for disease resistance) as it is not possible to select for resistance in the field in the absence of the pathogen. This resulted in the disease resistance of new hybrids either remaining constant or being more susceptible when compared to existing hybrids on the market.

Tester selection is important and could, if not correct, nullify much of the work conducted in selecting ear rot resistant material. Usually, the tester selected will be a proven inbred or single cross with good general combining ability that is likely to identify any quantitative variation in the material being tested. However, there may be cases when specific types of testers or testers of known backgrounds need to be used. The inbreeding stage of initial testing for yield will depend on the objectives of the programme and may also be governed by personal preference.

S. maydis ear rot resistance in South Africa is primarily quantitatively inherited but a wide range of genetic resistance is available. The range of resistant material and the type of resistance available provides adequate material for ear rot resistance breeding. This supports Boling and Grogan (1965) who found that the best method for selection for ear rot resistance was through reciprocal recurrent selection, although backcrossing could be used in the specific case they studied.

S. maydis is a necrotroph when causing a stalk rot (low sugar disease). However, the maize ears are large sinks for carbohydrates and *S. maydis* infecting ears is either a biotroph or hemibiotroph (high sugar disease). It is unlikely that a pathogen can be both a necro- and biotroph is the same plant and at the same time, causing two different diseases. Once *S. maydis* has infected the ear, in senescing cells of the shank and cob, the sink is severely affected as carbohydrates cannot get to the kernels as easily due to the colonisation of the shank and cob tissue. After this the ear is stressed and *S. maydis* becomes an efficient necrophytic feeder. The stage of infection, and tissue that is colonised, will determine the severity of ear rot at harvest; i.e., the time the pathogen has to develop and the site on the ear that the pathogen infects.

1.18 Screening

There is not necessarily a good correlation between ear resistance expression in artificially inoculated and naturally infected maize; only specific material correlates well, with majority of germplasm showing little to no correlation. This is due to the circumvention of the mechanical resistance mechanisms (Koehler, 1959; Kerr, 1965; Gulya *et al.*, 1980). It is of concern that artificial inoculation techniques are used as the rule, rather than the exception, in most of the research/breeding organisations in the USA and Europe. This is often because researchers using these inoculation techniques either do not test this material under natural conditions or not have the resources to undertake this work. An attitude that appears to be particularly strong in the USA is that there is so much useful resistance available after artificial inoculation, that the argument of the so-called "lost resistance" is not valid. However, with a severe selection pressure these researchers may in fact be selecting for highly heriditable vertical resistance (VR), which is not desirable in the long term. Unfortunately, the problem with natural infection is usually inconsistent inoculum levels within an experiment. Therefore, any method of naturally increasing the inoculum level to a higher, more consistent level, is beneficial.

Other than the lack of correlation between natural and artificial inoculation techniques, there appear to be a few other complicating factors associated with artificial inoculation. Biotypes are important in that there can be interactions between biotype and genotype in anthracnose and *G. zeae* stalk rot (Mesterhazy and Kovacs, 1986; White *et al.*, 1987). According to Chambers (pers. comm.) and Flett (pers. comm.), there is much variation between biotypes of *S. maydis* in South Africa. This immediately introduces the problems of which biotype to use, multiple inoculations, mixing biotypes in suspension and how to interpret the results. The combinations

are also likely to vary for each geographical area, or screening has to take place over a number of locations. There is presumably no cost effective means of doing this without error. Koehler (1959) stated that there can also be differences in aggressiveness in a biotype depending upon the age of the culture used.

If screening for ear rot resistance under natural epidemics is not possible, then essentially two methods are well accepted internationally. The first method is to inject a conidial suspension into the ear, and the second method is to inoculate with *Stenocarpella* colonised toothpicks, the latter technique being favoured for ear rot.

The method developed of naturally increasing *S. maydis* inoculum pressure in South Africa (Chapter 2) worked well and shows good correlation with the hybrid response to ear rot in the field. The effectiveness of this method has been verified by Bensch (1995) and is used as the standard method to screen germplasm by the Agricultural Research Council (Flett and McLaren, 1994). The only modification made is to inoculate with grain colonised by a pure culture of *S. maydis* instead of the milled *S. maydis*-infected ears from the previous season. This is desirable if the time, effort and cost in producing this pure inoculum can be justified. The method is ideally suited for use by a commercial organisation for screening large numbers of plants for ear rot resistance as it is simple to undertake, efficient, effective and in-expensive. The method of Warren and Onken (1981) of applying a conidial suspension would be effective but the inoculum is more difficult to produce and regulate, is sensitive to environmental conditions, culture age and subject to loss of virulence in culture.

Irrigation can play an important role in screening programmes in ensuring that *S. maydis* inoculations are effective and ear rot develops after inoculation. In most cases irrigation would be supplementary to rainfall to ensure the maize does not get too dry.

1.19 Assessment

A number of different methods of ear rot assessment have been employed in the past (Hoppe and Holbert, 1936; Koehler, 1959; Villena, 1969; Pappelis *et al.*, 1973; Gulya *et al.*, 1980; Rheeder, 1988). However, many of the methods used are labour intensive and slow in deployment. Results presented in Chapter 3 show that the assessment method used will largely depend on the desired accuracy and time available to undertake the assessment. The accuracy

would be determined by the testing phase of the germplasm being screened for ear rot response. Initial or preliminary trials could be visually assessed using a simple rating scale or index. More advanced trials would require an increase in the accuracy and the percentage of *S. maydis* infected ears would be determined. In commercial hybrid trials and trials where accuracy is important, the percentage of diseased grain would be determined. This is a time-consuming method of ear rot assessment but reflects the actual disease levels in the field as determined upon delivery of a grain crop to the storage silos. More detailed analysis of ear rot would be used for pathological studies only.

When using a rating scale it is important to realise that the rating gives an estimate of ear rot only and that this scale is often not linear in nature. This can give rise to incorrect information if these data are meaned arithmetically (Gulya *et al.*, 1980). This is an additional reason why actual percentage of ear rot should be used when trying to accurately determine the level of ear rot or germplasm response to ear rot.

In Chapter 3, experience in maize research is important when assessing ear rot. Researchers with less than 3 years field experience were inaccurate when assessing ear rot. This emphasises the need for training of inexperienced researchers in ear rot assessment. It is also important that researchers should "standardise" their ear rot assessments at the beginning of each harvest season. This should ensure more uniformity between researcher's assessments.

A factor that needs close attention is that of "hidden" ear rot. This form of ear rot is easy to underestimate and can make up a large proportion of the total kernels rotted. The only way to accurately determine the amount of "hidden" ear rot is to individually examine each ear. This can be very time consuming. "Hidden" ear rot is particularly important when infection and colonisation presumably takes place late in the season.

Although *S. maydis* ear rot is the most important ear rot in South Africa, a number of other fungi also cause ear rot. This is all taken into account when the percentage diseased grain is determined. When determining the resistance levels to *S. maydis* only, this would be undesirable. In this case, the most accurate assessment method would be to accurately determine the percentage of *S. maydis*-infected ears.

1.20 Fungicides

Considerable research has been done in Greytown, KwaZulu-Natal, to find fungicides that can be used to control *Stenocarpella* ear rots in seed production fields. Initial research with benomyl proved that ear rots could be controlled effectively with one or two fungicide applications, provided the fungicides were not applied by aircraft (Nowell, unpublished). Subsequent research proved that large reductions in the incidence of ear rot, and significant grain yield increases, could be obtained through the application of benomyl from 10 days before anthesis to approximately 3 weeks after anthesis. This work showed that fungicide application in seed crops is economically viable but would not necessarily be viable for commercial maize producers. Although the most consistent and effective fungicide was benomyl, the triazole group of fungicides also showed promise for *Stenocarpella* ear rot control. Although both knapsack and tractor application of the fungicides were effective, aerial application was not. This is likely due to the volume of water being applied as water volumes of less than 150ℓ ha^{-1} resulted in reduced efficacy of the fungicide. In addition, not all genotypes responded to fungicide application. Many factors can influence the efficacy and economic viability of fungicide application. These are:

i) the natural resistance level of the host,
ii) prevailing weather,
iii) growth stage at application,
iv) duration between applications,
v) method of application, and
vi) rate of fungicide applied.

Not only did the use of fungicides increase yields, but it also increased the fraction of the crop that could be sold as seed. This increased the profitability of fungicide use substantially for seed companies (Farwell, unpublished; Nowell, unpublished).

The use of fungicides to improve seed yield and quality needs to be developed further. In general, seed quality would improve substantially if fungicides were used to control diseases on susceptible seed parents. The profitability would consequently improve. The judious use of fungicides would allow normally high risk disease regions to be utilised for seed production. These high risk regions are usually regions of higher and more stable rainfall, which would reduce the chances of crop losses.

The use of fungicides on maize to control *S. maydis* ear rot would be limited to seed crops only. This means that these products would have to get minor use registration with the relevant authorities, a process easier than registration for a food crop.

Resistance by *S. maydis* to the fungicides employed to control ear rot is a potential problem. However, the fungicide is usually only applied once or twice a season and the chances of resistance building up to these products are low (Dekker, 1986; Delp, 1988; Wade, 1988).

1.21 Fertility

The practice in South Africa, particularly in the Highveld and KwaZulu-Natal, to let cattle graze on the maize stalks after the ears have been harvested has become a common practise. This reduces feeding costs and adds another dimension to the mixed farming enterprises. However, there may be a problem associated with this. Allowing cattle to graze on the stalks means that the farmers cannot till their fields until the cattle have finished grazing. Therefore, either the maize debris gets buried very late in the season, reducing the time available for breakdown of the debris, or the farmers introduce reduced tillage practices. Both methods increase the likelihood of increased ear rot the following season. A further interesting phenomenon is that the application of cattle manure to a field may influence the incidence of ear rot. A survey in a field near Greytown, KwaZulu-Natal, that had received two different rates of cattle manure during the 1987/88 season, revealed an increase in *Stenocarpella* ear rot with increased manure levels. However, there was also an increase in the number of healthy ears. This means that the increase in ear rot was not in the number of diseased ears, but rather in the severity of disease (Nowell, unpublished). This raises the issue of high yielding maize (those fields that received manure had a higher yield than those that did not) being less able to withstand the spread of ear rot once infection has taken place. This is theoretically possible on the basis that ear rot is a high sugar disease. These are further aspects that need investigation in association with fertility trials.

Jones and Duncan (1981) found that maize produced with nitrogen stress (low nitrogen) had significantly higher levels of aflatoxin B_1 ($\leq$240 times higher) than maize grown without nitrogen stress. Warren *et al.* (1975) found that there was a significant reduction in the incidence of *F. moniliforme* in the kernels with the addition of anhydrous ammonia alone or anhydrous ammonia with nitrapyrin to the soil. However, no information is available on

mycotoxins in *Stenocarpella*-infected ears that have been stressed through inadequate nitrogen stress. This needs to be investigated and would also be relevant to the *Fusarium* mycotoxins.

1.22 Biological control

An area that does warrant further research is that of biological control of seedborne fungi and subsequent root, crown and stalk rot. Promising results have been published at times (Kommedahl and Mew, 1975; Vakili, 1985) and commercial biological seed treatments are now becoming available; e.g. Kodiak® from Gustafson. No research has been undertaken on maize in South Africa. Soil additives and adding stalk rot mycoparasites to the whorl of the plants also warrant further research. Vakili[26] (pers. comm.) found that by adding mycoparasites from the stalks of the previous crop to the whorls of the maize plant at approximately the 10 leaf stage, there was a reduction in the incidence of stalk rot. Unfortunately, this project was terminated before final results could be obtained and no ear rot determinations were obtained.

1.23 Early drying

Artificial drying after early harvesting of maize can have a large effect on the incidence and / or severity of *Stenocarpella* ear rot. Research by Farwell (unpublished) showed that the optimum time to harvest seed maize is at physiological maturity (±40% grain moisture). However, there appeared to be differences between genotypes. These data are based on the visual assessment of diseased grain. The resistant genotype tended to reach maximum *Stenocarpella* ear rot approximately 28 days after physiological maturity, but a more susceptible genotype reached maximum ear rot about 24 days after physiological maturity. The rapid increase in diseased grain between 47% grain moisture and 29% grain moisture is significant to the seed industry. To get the benefit of early drying it is then important to harvest the crop as early as possible. Artificial drying of maize grain is not necessarily a viable proposition to the commercial producer, unless there is an additional bonus for early delivery or the delivery of superior quality grain. A further interesting phenomenon, is that of the differences between plant densities for the prevalence of ear rot with early harvesting. Lower plant densities resulted in a greater increase in ear rot with later harvesting in most seed parents tested. Plant densities above 44 000 plants ha^{-1} have almost a linear increase in disease with time. It is important that

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the relationship between internal seedborne fungi and the effects on seed germination, vigour and viability over time be studied further. It would be of interest to seed companies if early harvested maize stores better due to fewer internal seedborne fungi. Such studies would also allow the optimisation of seed treatments.

1.24 Mycotoxins

The effects of mycotoxins on animals are worrying, since in excess of 40% yellow grain delivered to storage silos had greater than 4% rotten grain during the 1987/88 and 1988/89 seasons. At least 15% of the yellow grain delivered in 1988 had >8% rotten grain and there are no records of the amount of sample graded yellow maize that was delivered or utilized as animal feed. There was significantly less rotten white grain during the same period. However, it is primarily the yellow grain that is used for animal feed. Although *Stenocarpella* ear rot does not affect cattle once shelled, the pig, poultry and duck industry cannot maximise utilisation of the yellow grain without significant effects on the animals. If the *S. maydis* and *S. macrospora* mycotoxins were identified, rapid and accurate means of determining feed value could be developed.

A further point touched on by Rheeder (1988) and Rheeder *et al.* (1990) was that of the effect of *S. maydis* infection on plant / seedling growth. Cutler *et al.* (1980a and 1980b) found that diplodiol, isolated from *S. macrospora*, caused selective growth responses in maize plants. This needs to be investigated further, but the problem is that the toxin produced by *S. maydis* has still not been characterised. Viljoen *et al.* (1994) showed that normally *S. maydis* infected maize is in the minority and the major effect of *S. maydis* would be in the second and third grade maize. This means that the mycotoxins produced by the various *Fusarium* spp. are more important in maize overall.

2 GREY LEAF SPOT

Grey leaf spot was first noted by South African scientists when it appeared in South Africa in the late 1980s. However, it now transpires that the disease has been present in other African countries for about the same period of time. According to Pixley[27], GLS has been present in

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Uganda for at least four years and is causing severe yield losses. Farmers and extension personnel in Cameroon maintain the disease has been present in their countries for at least six years and is also causing large economic losses. Zimbabwe officially reported the pathogen for the first time during 1996. This pathogen has become a problem in Africa and is fast becoming an international problem. Research on GLS in South Africa, under African conditions, can be used effectively by other African countries to find solutions to GLS in maize.

The impact of GLS on the farming community in the early 1990s in KwaZulu-Natal, South Africa was large and the continued production of maize in this region was threatened. A feature of GLS is the rate at which it spreads and establishes itself. Consistent yield losses in excess of 30% (Tables 8.1 - 8.4 and Table 8.9) in South Africa, on susceptible hybrids, cannot be absorbed in commercial farming enterprises. Grain yield losses of 2.390 - 3.504 t ha^{-1} or a financial loss of R1 554 - R2 278 ha^{-1} were reported for the 1992/93 and 1993/94 seasons. Such economic losses justify urgent and significant research efforts to determine the most cost-effective control measures under South African conditions.

2.1 The pathogen

Although the life cycle of *Cercospora zeae-maydis* Tehon & Daniels has been studied, there are a number of areas that need further research, particularly under southern African conditions. In the literature there is some confusion as to the species of *Cercospora* that causes GLS on maize (Hyre, 1943; Mulder and Holliday, 1974; Shurtleff, 1980; McGee, 1988). A study is needed using a number of different biotypes of *C. zeae-maydis* to determine whether maize is the only host. It is also important to determine whether *Cercospora sorghi* can infect maize. A wide range of the Gramineae should be tested as possible hosts as this would be important in the life cycle of the pathogen.

Considerable variation in the size of the conidia has been reported (Tehon and Daniels, 1925; Chupp, 1953; Kingsland, 1963; Latterell and Rossi, 1983b; Levy[28], pers. comm.). This needs to be clarified, particularly if it were true that different species are involved or alternate hosts are available. Bair and Ayres (1986) noted considerable natural variability in the pathogen in the field. This suggests that much of this variation may in fact be natural. No comparative

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studies have been undertaken to compare biotypes and / or pathotypes from the Americas, Asia and Africa. These studies should be based on pathogen morphology, isozyme patterns, DNA fingerprints and host response to the biotypes. A collaborative programme on this is planned for early 1997 and will involve pathologists from South Africa, the USA and Zimbabwe. Other scientists and countries will be drawn into this programme. This would also give insight into where the pathogen originated and how it spread around the world.

The teleomorph of *C. zeae-maydis*, a *Mycosphaerella* sp., was briefly mentioned by Latterell and Rossi (1983b) and was said to be insignificant in the initiation of epidemics. This may well be the case in North America, but in the sub-tropical and tropical environments the teleomorph could play a significant role in the life cycle of the pathogen. The teleomorph would play an important role in long-term survival of the pathogen, initial infection, long distance dispersal, rapid spread of the pathogen and result in a significant amount of genetic variability in the pathogen population. This would have significant impact on GLS resistance breeding and fungicide usage strategies. As an example of the different sexual phases being prominent in different crops and regions, the anamorph of *Leptosphaeria maculans* (Desm.) Ces. & deNot. is central in blackleg of cabbage. However, the teleomorph of *L. maculans* is predominant in canola in causing blackleg (Laing, 1996).

2.1 Distribution

Figure 2 shows the worldwide distribution of GLS as of January, 1996, and is based on Boothroyd (1964), Latterell and Rossi (1983b), Ward *et al.* (1993), Coates and White (1994) and Nowell (unpublished). Initially the GLS epidemics in South Africa were localised to the KwaZulu-Natal region but with the increased distribution of the disease in the Mpumalanga province, GLS has now becoming a national problem. Bensch and Flett[29] (pers. comm.) reported that GLS has now spread to the Amsterdam, Amersfoort and Wakkerstroom regions, and have also found it in the Brandfort, Vrede, Reitz, Harrismith, and Bethlehem areas of the maize production region during the 1995/96 growing season. The disease has also been noted at Jozini, northern KwaZulu-Natal, and Komatipoort, eastern Mpumalanga. Unconfirmed reports have been received from an even wider area than this. This is highly significant, as a

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major portion of the maize production region in South Africa in now threatened should environmental conditions be conducive for infection and disease development. It is expected that with time, GLS will be endemic throughout the South African maize production region. However, GLS epidemics will be limited to the eastern maize production regions with a higher rainfall and cooler summers. Certain areas in the eastern Highveld region (from Piet Retief to Carolina and Lydenburg) can expect to experience economic losses during years of normal rainfall, once inoculum has built up in this region. The area of Boons and Caltonville region (west of Gauteng) could be a hot spot for GLS as the rainfall in this region is slightly higher than surrounding areas. However, should wet seasons follow each other, the likelihood of a GLS epidemic in other regions is much higher, particularly in the low-lying valleys. This would be very similar to the GLS disease pattern in the USA (Stromberg, 1986; Anderson, 1995).

Grey leaf spot has been positively identified (Figure 3) in Zimbabwe, Cameroon, Uganda, Malawi (southern Malawi and Mzuzu), Zambia (from the Mkushi Block to Kasama), Zaire (Lubumbashi region), Kenya (Eastern Highlands), Mozambique (southern region), Swaziland and Tanzania (Mbeya). The severity of GLS in Cameroon, Uganda and Zimbabwe is of concern to both maize farmers and researchers. Figure 4 shows the known distribution within Africa as of December 1996. GLS has been noted in specific regions of the above countries and is likely to be present throughout the countries to a greater or lesser degree. Based on the known distribution of the pathogen in Africa, meteorological data and topography of the region, it is likely that the pathogen is also present in, or will be in the near future, Angola, Burundi, Central African Republic (western region), Chad (southern region), Congo, Ethiopia (highlands), Gabon, Nigeria, Rwanda and Sudan (southern region). The distribution in West Africa is unknown but the environment in the region is favourable for GLS epidemics.

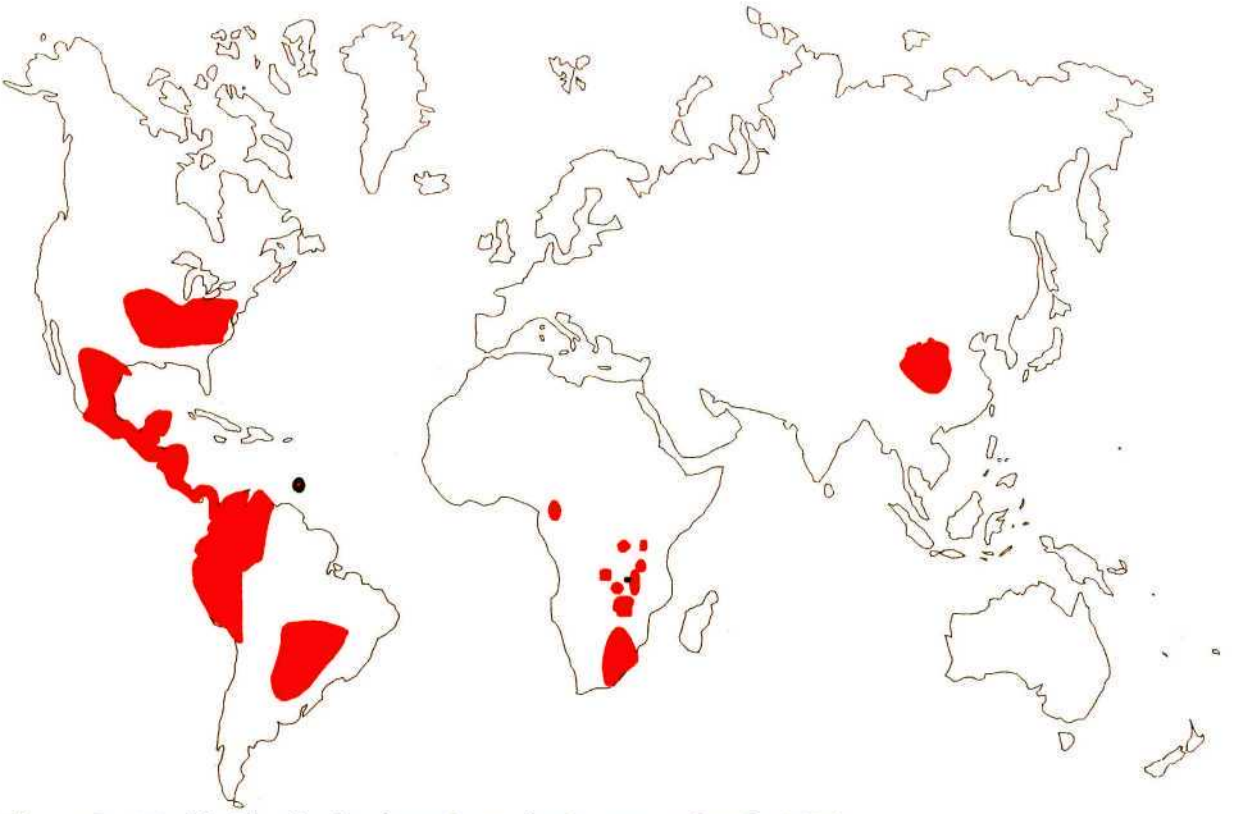

Figure 2: Worldwide distribution of grey leaf spot as of early 1996.

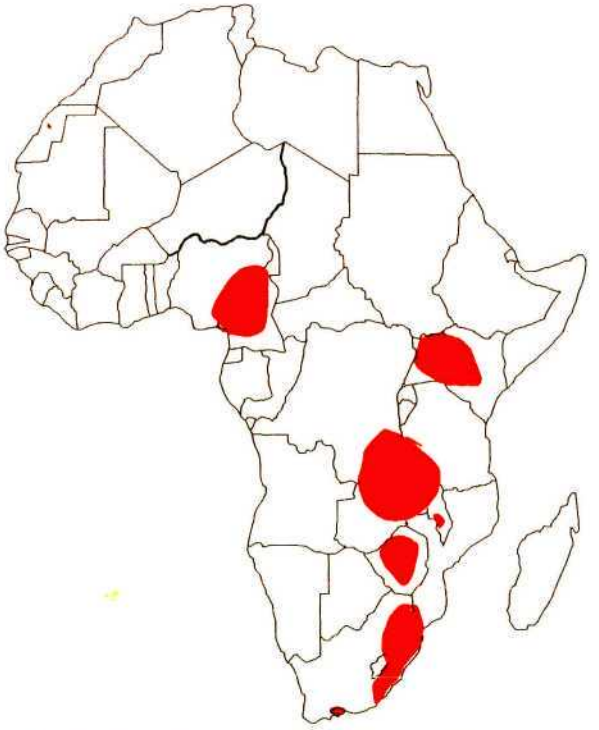

Figure 3: Present distribution (December 1996) of grey leaf spot in Africa.

Figure 4: Proposed distribution of grey leaf spot in Africa.

The development of GLS in Zimbabwe this past season was similar to the disease pattern experienced in South Africa during the first few seasons of its occurrence. Similarly, in South Africa, GLS took three seasons after it was first identified before significant yield losses occurred regularly. A number of Zimbabwean researchers and farmers are adamant that *C. zeae-maydis* has been present in Zimbabwe for at least three seasons. A local seed company first noticed the disease in their breeding nurseries during February 1995. GLS became problematic in the Marondera and Mwurwi areas in January and February 1996 and grain yield losses in isolated cases were over 50%. Once researchers and agronomists became aware of the symptoms, many reports were received from around the country. A large proportion of the maize last season was lightly diseased or disease occurred late in the season. However, the pathogen has now established itself and should environmental conditions be favourable for the disease, significant epidemics can be expected in previously unaffected areas from 1997 onwards. Climatology maps for Zimbabwe show that much of the country has temperature and moisture conditions favourable for the occurrence of GLS during the summer months. Early indications are that GLS is more widespread and has started to develop about one month earlier than it was in 1996 (Cowley[30], pers. comm.). Only a limited number of areas in Zambia have been examined for GLS, but it appears to be following a similar pattern to Zimbabwe.

During a visit to Cameroon and Kenya in October 1996, the GLS epidemics observed were severe. However, little was known by the maize researchers or agronomists about the disease or the effect it had on grain yield. Often people thought the maize was just drying off earlier than expected. Great concern was expressed when estimated grain yield losses were given. It is suggested that GLS is either already present in any of the African countries or will arrive in the near future (Figure 4), yet they are badly prepared for this eventuality. This is usually due to a lack of expertise, knowledge and resources to do anything about such a disease. South African knowledge will have to be drawn on heavily to help combat the effects of GLS.

The sudden appearance of the pathogen in South Africa in 1988 gave rise to many theories as to where the pathogen originated and how it arrived in South Africa. As the pathogen is not considered to be either seed-borne or seed-transmitted (McGee, 1988), these possibilities were not considered options in the pathogen being introduced into the country. The most common and plausible theory, is that the pathogen arrived on imported *C. zeae-maydis* surface

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contaminated maize grain intended for human consumption and animal feed, and/or *C. zeae-maydis* infected maize debris in grain from the U.S.A. in the mid-1980's (Cronje, pers. comm.).

The fact that the pathogen is relatively widespread in southern / central Africa, suggests that the pathogen in South Africa may have originated from this region rather than being the source of infection for the sub-continent. The mass of warm, moisture air called the Inter-Tropical Convergence Zone (ITCZ), which moves from the central, tropical African region to the southern African region in the southern hemisphere's summer months, and back again in winter, could have aided the dispersal of the pathogen to South Africa from southern / central Africa. It has been suggested by others that the ITCZ could be responsible for the spread of pathogens (Robinson, 1976; Vanderplank, 1984). The long distance spread of wheat rusts in North America and India (Zadoks and Schein, 1979), and the dispersal of blue mould of tobacco (caused by *Peronospora tabacina* Adam) from the Caribbean region through North America show that long distance dispersal within a continent and within a season is possible (Davis and Main, 1986). The spread of *Hemileia vastatrix* Berk. & Br. of coffee and *Puccinia melanocephalia* H. & P. Sydow of sugarcane to continents previously free of these diseases, was suggested to be via trans-Atlantic wind currents (Purdy *et al.*, 1983; Schreiber and Zentmeyer, 1984).

Little research has been undertaken on the dispersal of *C. zeae-maydis*. Comparative studies are needed under a number of different types of weather systems. Observations in KwaZulu-Natal, where the disease was first observed, showed that the pathogen could spread over 300 kilometres in a single growing season and cause a significant number of lesions in the same season. Research on dispersal should include both conidial and ascospore (possible teleomorph) dispersal on a micro and macro scale. Epidemics regularly occur on maize fields that have not been cropped to maize for more than five years. Many farmers (particularly in Zimbabwe) have reported most of the disease in the canopy above the ear in the first season. This suggests wind dispersal from outside the diseased crop.

There is likely to be a high atmospheric concentration of conidia in the region of *C. zeae-maydis-infected* maize due to wind-borne dispersal of the pathogen. The question then arises of whether aircraft can play a role in long distance dispersal of *C. zeae-maydis* conidia. Conidia could be present in the air within the aircraft or the air, or mechanisms, within the undercarriage. Should this be possible, this is a way of dispersing new biotypes or introducing

GLS to regions previously free of the disease. The chances of this happening are small but tangible.

2.4 Rotation

As this pathogen does not survive much beyond a season on debris colonised by *C. zeae-maydis*, rotation has been recommended as a control measure or an alternative to ploughing (Latterell and Rossi, 1983b; Stromberg, 1986; Spink and Lipps and 1987; Huff *et al.*, 1988; Ward *et al.*, 1993). Although rotation is likely to have significant agronomic benefits (Palti, 1981), the specific benefits of a rotation system to control GLS have not been quantified. Due to the windborne nature of this pathogen, it is unlikely that maximum benefits will be obtained from a rotation system. The main benefits are likely to be a delay in the onset of the disease (as the inoculum source is outside the field) improved nutrient status and a reduction in maize soilborne pathogens. The duration of this delay in onset of disease will be largely influenced by the prevailing climatic conditions. A rotation trial, that had originally been designed to determine the effect of rotation on ear rot, was examined for GLS differences. Unfortunately, the plots were too small for a windborne pathogen and as a result there was significant interplot interference, minimising benefits (Nowell, unpublished). Trials are currently underway to determine the effect in the KwaZulu-Natal Midlands and in the south east Mpumalanga province.

2.5 Tillage

Plant debris from the previous season is the only source of inoculum for subsequent maize crops. This means that any form of debris reduction or elimination is a desirable practice that will reduce the inoculum level for subsequent maize crops. For this reason, tillage practices that bury debris and crop rotation are the most important cultural control measures. However, it is important to realise that this will not eliminate the disease but only delay the onset of GLS. Due to the windborne nature of this pathogen, conidia can originate from a considerable distance from a maize crop and cause an epidemic that would result in yield loss. For maximum effect, rotation and incorporation of debris has to be practised over as larger an area as possible. The type of tillage practised is also very important as ideally all debris should be buried at least 10 cm below the soil surface. It has been shown for *S. maydis* that partially buried debris is able to produce a large number of conidia as the plant material is in close contact with the soil moisture (Flett, 1990).

Research on the survival rate of *C. zeae-maydis*-infected plant debris has been conducted in the USA (temperate climate). It is important that this research be repeated under local conditions as the climatic conditions are different, especially in winter. The research by Payne and Waldron (1983) suggested that the survival rate of debris in the mountainous and plain regions of North Carolina are significantly different. Differences in climate between the USA and Southern Africa would be even greater. This information is necessary to optimise tillage and rotation practices under local conditions. It would be useful to repeat this research under tropical conditions too.

Different tillage practices need investigation under local conditions. The differences between autumn and spring ploughing is likely to be greater under sub-tropical conditions compared to temperate conditions, as the winter months are relatively mild and microorganisms would be active in debris breakdown. Reduced tillage methods needs to be studied and possible methods of enhancing debris breakdown investigated.

The advantages and disadvantages of the various tillage methods need to be balanced against benefits to the farmer of the common practice in South Africa of allowing animals to graze plant debris during winter. Grazing of the maize debris by animals alone needs to be investigated to determine whether or not this has an effect on the GLS inoculum the next summer season.

2.6 Burning

Burning of plant debris is generally an undesirable practice that reduces organic matter of the soil and allows excessive water runoff and erosion during rainfall. As *C. zeae-maydis* is a windborne pathogen, an external inoculum source is adequate to cause a GLS epidemic. Burning would have limited effect in reducing GLS. The negative factors associated with burning far outweigh the potential benefits.

2.7 Silage

Harvesting of maize for silage is essentially removing infected debris or inoculum from the field. The affect on GLS incidence the following season will be similar to practising rotation and/or ploughing the field. The benefits of removing maize stover as silage need to be quantified. However, the use of maize as silage raises a number of questions:

i) Do fungicides applied during the growing season continue to breakdown once silage has been cut, and are the normal withholding periods adequate?

ii) At what stage does GLS begin to affect the quality of the silage?

iii) Are any quality factors, such as protein content, affected?

iv) Is the silage yield affected significantly?

These questions need to be investigated as a number of farmers in KwaZulu-Natal are cutting fungicide-treated and *C. zeae-maydis*-infected maize for silage. This information would allow farmers to optimize the use of silage.

2.8 Seed treatments / germination / vigour / seedling diseases

McGee (1988) states that there is no evidence or records of *C. zeae-maydis* being seed-borne or seed-transmitted. This may be true but a number of factors suggest this information needs to be checked. There are many of other *Cercospora* spp. that are seed-borne (Richardson, 1990) and *C. zeae-maydis* may not be an exception. This fungus grows slowly on artificial media and can be difficult to isolate or obtain a pure culture (Beckman and Payne, 1983; Latterell and Rossi, 1983b). Therefore, conventional methods of isolation may not be adequate as the fungus would not be a good competitor with other organisms found in or on seed. Either selective media or the new DNA technology could be used to confirm that this pathogen is not seed-borne.

As *C. zeae-maydis* is not considered to be seed-borne or seed transmitted (McGee, 1988), no phytosanitary regulations regulate this pathogen. Should this have been a factor, such measures would be ineffectual due to wind dispersal of this disease.

Maize grain has a significant amount of plant debris present on the seed surface after harvest. *C. zeae-maydis* conidia may be on the seed surface as contaminants or on the little bits of plant debris in with the grain. The pathogen could easily be transmitted to a disease free region in this way (as postulated to be the method of introduction into South Africa). The presence and viability of such inoculum should be investigated, particularly on imported maize grain.

During the droughts in the early 1990s, the farmers from the Free State, North West, Gauteng and Mpumalanga provinces baled and transported *C. zeae-maydis*-infected debris to these areas to feed their animals. Fortunately the next season was not unduly wet and GLS did not appear

establish itself. Subsequently two consecutive wet seasons have seen the disease moving into these regions. The significance of the infected debris taken to these regions cannot be accurately determined. It has also been hypothesised by the Maize Board that this is in fact how GLS first arrived in South Africa (Cronje, pers. comm.).

2.9 Planting date

The effect of planting date will vary between regions. In general, the period of the season with the highest rainfall will result in the highest incidence of GLS. Therefore, planting to avoid this presumed peak infection period would be beneficial, provided yield potential and yield reliability are not compromised. In KwaZulu-Natal, the earlier maize is planted, the better as the months of December, January and February are most favourable for *C. zeae-maydis* infection and development. Planting very late will result in lower yields as the rainfall is not as reliable as earlier in the season. However, planting too early is likely to result in cold / frost damage to the crop.

2.10 Infection

It is unusual for a germinated fungal spore to survive dry periods and for infection to take place in the absence of free moisture. The powdery mildew fungi are the only fungi, other than *C. zeae-maydis*, that do not require free water on the leaf surface to successfully germinate and penetrate the leaf surface, either directly or via stomata (Boothroyd and Roberts, 1984). Since high humidity occurs more often than free water (usually dew), this adaption by *C. zeae-maydis* represents a very significant environmental adaptation, with important epidemiological implications. Studies on germination, the infection process and subsequent development of *C. zeae-maydis* have shown that the fungus is able to withstand considerable variation in the climatic conditions over this period. Once germination of the conidium has been initiated, the germtube is able to withstand periods of relatively dry weather without dying (Rupe *et al.*, 1982; Thorson and Martinson, 1993). This means that the cumulative hours of high humidity are important, rather than a specific continuous period of high humidity. Penetration and infection can then take place over a longer period of time under conditions that are less than ideal. In this respect, the pathogen is unique amongst the maize fungal pathogens.

Once the fungus has established itself in the leaf tissue, it is able to enter a dormant phase

should conditions be unfavourable for disease development (Latterell and Rossi, 1983b; Stromberg, 1986). The fact that infection and colonisation is apparently a lengthy process, taking 7 - 9 days, is in some ways in conflict with the fact that this disease can increase from 5% to over 75% leaf area loss in approximately three weeks. The first signs of sporulation usually appear about 9 - 11 days after infection has taken place. The various parameters in these processes need to be determined under local conditions, both in the field and under laboratory conditions. The quantity of conidia produced per unit area of lesion and duration for which each lesion can produce viable conidia need to be determined on very susceptible, moderately susceptible and resistant germplasm. The early dynamics of the epidemic would then be better understood, allowing for a better understanding of the interaction between climatic conditions, early disease establishment and development of the disease. This would allow for the optimisation of possible control measures early in the disease cycle and any mathematical models that may be developed for GLS. An added complication is that there may be significant differences between biotypes of *C. zeae-maydis*, particularly between the tropics, sub-tropics and temperate regions.

Temperature appears to be more important than moisture in *C. zeae-maydis* infection and subsequent development of the fungus. During the 1992/93 season, rainfall was limited at Greytown in KwaZulu-Natal, although dews were frequent, but a GLS epidemic still occurred. During the 1995/96 season, some areas of KwaZulu-Natal had adequate moisture but unusually low temperatures (maximum of 20°C or less) and GLS was slow to develop. An in-depth study is needed to determine the exact climatic factors affecting *C. zeae-maydis* under local conditions. A number of weather stations are situated in the areas where GLS is endemic that record rainfall, maximum and minimum temperature, evaporation, sunshine hours and wind distance (and sometimes wind direction) which could be used for this purpose. The emphasis would have to on collecting accurate field data on the incidence and severity of GLS.

Rupe *et al.* (1982) found that free moisture on the leaf surface reduced stomatal tropism, that appressorium formation was rare and host tissue was not penetrated. This could play a role in the sub-tropical and tropical region where GLS occurs as the fungus may not be able to establish itself effectively during periods of prolonged and frequent rainfall. This needs investigation in these regions.

2.11 Symptoms

There is concern about the variation in symptom expression on maize hybrids in the USA (Coates and White, 1995; Pataky [31], pers. comm.) and in South Africa (Ward[32], pers. comm.; Nowell, unpublished). An example of this in South Africa are the lesions which develop on the hybrid PAN 6480 which are normally small, severely restricted lesions, but which can become normal large lesions towards plant maturity. The reason for this is not known. Often both lesion types have occurred next to each other on the same leaf. There is apparently a large variation between the frequency of these large lesions on this widely planted and resistant hybrid. Maize breeders need an explanation for this so that changes can be made to assessment and breeding strategies if necessary. Possible reasons for this, which would have to be investigated, are:

i) That different species of the pathogen result in the different lesion types
ii) Different biotypes of the pathogen exist resulting in a range of symptoms
iii) The different lesions types are the result of the anamorph and teleomorph stages of the fungus
iv) Plant maturity influences symptom expression and / or different resistance mechanisms at different growth stages or physiological development result in different types of lesions
v) A pathogen and temperature interaction occur (this is known to occur in other crops such as wheat [Vanderplank, 1982; Vanderplank, 1984]) resulting in such hybrids being susceptible at certain temperatures when the resistance is no longer effective
vi) The concentration of sugars / carbohydrate present in the leaf at the stage of infection and subsequent disease development could influence resistance expression.

Ascochyta pinodes (Berk. & Blox.) Vestergr. on peas (*Pisum sativum* L.) also causes two distinct symptoms on the leaves, a fleck or a zonate lesion. Although both lesions can occur on the same leaf, the fleck lesions are usually limited to the more resistant, upper portion of the plant canopy, and the zonate lesions to the more susceptible lower portion of the canopy. The

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exact reasons for the differences in lesion type are not known but resistance appears to play a role (van Schoor, 1990).

2.12 Stress

Moisture stress, as observed during the 1992/93 season at Greytown and Cedara, does not appear to significantly influence the host susceptibility to the pathogen. It gave rise to a greater amount of variation within the ratings of hybrids, but the relative ranking appeared to be similar.

Fertility stress is considered to play a role in reducing the amount and severity of GLS (Farina[33], per. comm.; personal observations). The exact effects of the various elements and soil acidity is not known, but are in the process of being determined by Farina and colleagues at Cedara Agricultural Development Institute (CADI).

2.13 Plant density

High plant densities apparently reduce the severity of GLS (Smith, 1989; de Nazareno *et al.*, 1991; Carrera and Grybauskas, 1992; de Nazareno *et al.*, 1993a and 1993b; Rivera-Canales, 1993). This may be one of the reasons GLS is so severe in KwaZulu-Natal. There are large difference between the plant densities of commercial maize in South Africa and that in the USA. For this reason it is important to establish the effects of plant densities from 12 000 - 80 000 plants ha^{-1} as planted under conditions in South Africa. Plant densities of less than 35 000 - 45 000 plants ha^{-1} are those employed in the high risk GLS areas by South African farmers. Spore deposition rates and light penetration could be determined at the different plant densities.

To get a better understanding of the effect of light on GLS development, a simple experiment could be designed. Uniform, artificial inoculation of plants in pots in the glasshouse would ensure uniform infection of plants. They could then be transferred to maize plots at different plant densities in the field, where disease development and light penetration could be determined. This would indicate the effect that light concentration has on cercosporin and the development of the disease at the different plant densities. This could be supported by research in growth

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chambers at various light concentrations.

2.14 Irrigation

Ward *et al.* (1993) were the first to cite irrigation as a factor in GLS epidemics. The present author has seen maize fields irrigated by a centre pivot and drag line irrigation systems that have experienced major GLS epidemics, whereas maize not reached by the irrigation water in the same fields, had less severe GLS. This was particularly true during the drought of 1992/93. Rotem and Palti (1969) found that irrigated crops have more shade (due to fuller shoot development), lower temperatures, and longer periods of high moisture in the lower foliosphere and the upper soil layers. McLaren[34] (pers. comm.) has found that centre pivot irrigation, in a warm environment in South Africa, can reduce atmospheric temperatures in the crop by as much as 5°C at the centre of the pivot, when compared to the ambient temperatures outside the irrigated area. In maize, this would provide moisture for infection and reduce the temperature to levels more suitable for infection and disease development. This was apparent in the Greytown area in 1992/93, when the maize on the outside of a centre pivot irrigation scheme had 30% leaf area loss due to GLS, whereas maize in the centre had in excess of 70% leaf area loss.

2.15 Herbicides

Martinson *et al.* (1994) mentioned that trials had to be abandoned in Iowa because of an interaction between fungicides and a hormonal herbicide causing damage. The exact details of the circumstances are not known. The hormone herbicides (2,4-dichloroxyacetic acid in particular) have been studied intensively. However, there is much that is not understood about these herbicides which act as artificial auxins (Que Hee and Sutherland, 1981), which are plant hormones. Auxins interact with two other groups of plant hormones, the gibberellins and cytokinins. There is both synergism and competition among these hormones in the control of the physiology and development of plants (Raven *et al.*, 1981), including disease resistance responses (Haberlach, 1978).

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The effects of 2,4-D include:

i) increased disease susceptibility (Aberg and Stecko, 1976; Haberlach *et al.*, 1978; Hodges, 1978, 1980 and 1984)

ii) increased insect susceptibility (Maxwell and Harwood, 1958; Aberg and Stecko, 1976)

iii) increased incidence of nematode infestation of treated plant roots (Webster, 1967)

There are frequent cases of farmers reporting herbicide damage to maize crops in South Africa, but nobody has tried to find out if there is any subsequent effect on disease development. Although this type of research would likely receive a low priority rating, this type of information could be beneficial to farmers.

2.16 Resistance

Resistance is going to provide the long term, and hopefully permanent, control measure of GLS. For this reason, it is vital that the correct options are selected in identifying resistance, breeding methodology, screening germplasm and making this germplasm available to the farmers. Although much research has been undertaken on GLS resistance in the USA, many questions are as yet unanswered, particularly with regard to local conditions. It is presumed that resistance to GLS will be equally effective throughout South Africa as there appears to be a single pathotype of *C. zeae-maydis* that originated in KwaZulu-Natal and is now spreading throughout the maize production region. It is not known whether resistance to GLS varies between African countries or regions. Studies are underway to test the same set of hybrids in South Africa, Zimbabwe, Zambia, Kenya, Tanzania, Cameroon and Nigeria. Unfortunately, sending maize seed to the USA is prohibited unless as a quarantine phase in containment glasshouses is possible. This rules out sending hybrid seed to the USA. A possible solution is to obtain a set of hybrids from the USA for testing for GLS response under local conditions.

Ideally, only horizontal resistance (HR) to GLS should be released into the market place. This is unlikely, as Gevers and Lake (1994) and Gevers *et al.* (1994) have already identified a major gene for GLS resistance which is highly effective under local conditions. However, single or major genes for resistance do not necessarily imply that resistance will be VR in nature. Gene action is more important than gene number (Vanderplank, 1978 and 1984; Robinson, 1987). Most of the resistance employed against *C. zeae-maydis* in the USA is additive in nature but

there have been reports of major and single genes conferring resistance to the pathogen (Elwinger *et al.*, 1990; Coates and White, 1995). To ensure that resistance is HR, a single pathotype should be used for screening purposes and all immune material should be discarded. Testing with multiple pathotypes can lead to VR developing as there may be quantitative differences in the levels of parasitism even although all the hosts are matched. This is known as the Parlevleit effect and should be avoided (Robinson, 1987).

A recurrent selection programme is then necessary to ensure levels of GLS resistance are improved with each breeding cycle. Yield potential should be tested at each cycle of selection to ensure GLS resistance is not selected in lower yielding germplasm. During the selection process, agronomic factors must be taken into account to ensure the end product is acceptable to the farmer.

Some resistance sources are more complex than originally thought. Ulrich *et al.* (1990) found that certain inbreds conferred more resistance than expected when crossed to certain other inbreds. This made the prediction of resistance levels difficult but is of benefit in the ensuing hybrid. Such resistance will not respond well to a backcrossing breeding programme. Gevers[35] (per. comm.) has suggested that when some major genes are backcrossed into susceptible germplasm, they apparently do not always behave as a single gene. This makes GLS resistance breeding more complex than expected. It is interesting to note that in the traditional dent germplasm from the USA and Europe, little highly resistant germplasm is available. Studies to date have shown that South African GLS-resistant germplasm is generally more resistant to GLS than GLS-resistant germplasm from the USA (Nowell, unpublished).

A considerable proportion of the germplasm originating from the tropical maize production regions has useful levels of resistance to GLS. This resistance needs to be incorporated into high yielding commercial hybrids. This process will be slow as a small pool of germplasm that is GLS-resistant, high yielding, stable in different environments and agronomically acceptable. A good source of resistance needs definition as good usually implies high levels of resistance (= VR?) and may not be desirable in the long term. Inclusion of some of these resistance sources have already started to take place. However, it is important that research be continued

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to identify sources of horizontal resistance to GLS that can be used in conventional commercial breeding programmes.

The introduction of GLS-susceptible hybrids into regions that currently do not have GLS would enhance and aid the establishment of GLS and development of epidemics. Such a region is the North West province of South Africa. Although this region would usually not be environmentally friendly to *C. zeae-maydis*, certain areas are likely to eventually have GLS endemic. A major advantage at present is the fact that this region predominantly produces white maize which has acceptable levels of GLS resistance. Should GLS-susceptible hybrids be introduced on a large scale, GLS will establish itself sooner and be more severe than currently expected.

The same principle applies when replacing open pollinated varieties, traditionally grown by small-scale farmers, with high yielding maize hybrids in Africa where GLS occurs. A number of local African varieties and land races have high levels of resistance to GLS, but poor yields. Any hybrid or open pollinated variety that replaces these low yielding land races should have GLS resistance levels that are the same or better than those varieties being replaced. If this is not done, they could enhance a GLS epidemic in the long-term. This would essentially be as a result of an inoculum buildup. Grain yields would then decrease from the original highs when the hybrids were first introduced.

2.17 Inoculation methods / screening

The method employed for screening for resistance in a breeding programme can determine the long term success of such a programme. Firstly, it is important to have a consistent GLS epidemic (natural or artificial) that will allow good differentiation within and between germplasm. However, the severity of the epidemic must be such that a good range of quantitative resistance is identified. Consistent, severe epidemics will mask useful sources of resistance and favour the selection of highly resistant material. The danger in this is that it will skew the selection towards qualitative resistance. Vertical or quantitative vertical resistance is not durable and would only provide a short term solution.

For screening purposes, a GLS epidemic can be enhanced by irrigating the crop (wetting is sufficient) in the evening and early morning in order to extend the period that the leaves are

moist and the relative humidity is high within the canopy. GLS will develop rapidly if the disease is endemic. Artificial inoculation could be undertaken immediately after the plants are irrigated. The application of a conidial suspension (Latterell and Rossi, 1983b; Jenco, 1995) or *C. zeae-maydis*-infected plant debris should enhance a GLS epidemic.

2.18 Genetics

New molecular techniques allow for rapid inclusion of GLS resistance into previously susceptible germplasm (Bubeck *et al.*, 1993; Saghai Maroof *et al.*, 1996). This technology can be used to trace any number of genes with a great degree of accuracy. The above authors have determined multiple quantitative trait loci (QTL) which results in a broad degree of resistance, hopefully HR, to GLS. They have also shown that by combining backcrossing methodology with marker-assisted selection or specific random fragment length polymorphism's (RFLP's) technology, it is possible to transfer these resistance genes into susceptible inbreds. They also found that these QTLs appeared to be closely linked to resistance to other maize diseases. This may lead to unintentional associated benefits for the plant breeders. A problem with this technology is that some QTL's appear to have limited expression across environments (Bubeck *et al.*, 1993; Bohn *et al.*, 1996; Miklas *et al.*, 1996; Tuinstra *et al.*, 1996; Veldboom and Lee, 1996a and 1996b) and that these markers are not necessarily transferable across genetic backgrounds (Hookstra and Walton[36], per. comm.; Stuber[37], per. comm.). Only those markers stable across environments could then be used and each time a new genetic background is studied, the process of identifying QTL's has to start again. This type of research and technology is suited to maize breeding programme based on narrow genetic backgrounds, as is the case in the USA. This technology would be less suited to African maize breeding programmes, as very little high yielding germplasm is common between the two continents.

Saghai Maroof *et al* (1996) found GLS quantitative trait resistance loci in chromosome regions that are known to have a concentrations of other disease resistance genes, particularly to *Exserohilum turcicum* (Pass.) Leonard & Suggs and *Cochliobolus carbonum* Nelson. Studies in South Africa, have shown that although there does not appear to be a direct linkage between

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GLS and *E. turcicum* resistance, germplasm highly resistant to *E. turcicum* has a higher frequency of resistance to GLS. This is particularly noticeable in tropical germplasm. Possible linkages and interactions need to be investigated further. Genetic mapping provides a good tool to undertake this work.

2.19 Hybrid response

Considerable variation in hybrid response exists in South Africa. In general, the white-grained hybrids have higher levels of resistance to GLS than the yellow-grained hybrids, although both resistant and highly susceptible germplasm is present in both types of grain. Fortunately, approximately half of South African maize is white-grained and of this, a large proportion is planted to GLS-resistant hybrids. Most of this grain is produced in the lower yielding, western regions of the maize production area. Yellow-grained maize is largely produced in the eastern regions of the maize production region, where GLS is already endemic or expected to be endemic in the near future. A large proportion of this maize is susceptible to GLS.

When GLS first became epidemic in KwaZulu-Natal, the highly susceptible yellow hybrids RS 5206 and PAN 6552 accounted for the bulk of the plantings in this region. Within three seasons, these hybrids had been completely replaced with PAN 6480 (yellow-grained) and PAN 6479 (white-grained). Although their levels of GLS resistance were not high enough to eliminate grain yield losses, yields under GLS were higher than the old hybrids and the use of fungicides greatly reduced.

From ongoing hybrid evaluation trials, it is apparent that a significant proportion of newly released hybrids have improved levels of GLS resistance, particularly in the white-grained hybrids. This improvement should continue until there are hybrids available that will no longer exhibit grain yield losses due to GLS. Due to the nature of the climate in South Africa, not all hybrids released will have to be resistant or immune to GLS, as a large portion of the maize production region has a low rainfall (<500mm annum^{-1}) and has a hot climate. The climate is likely to limit the spread and severity of GLS in these regions, and susceptible or partially GLS-resistant hybrids can be cultivated in these regions. From a plant breeding perspective, it does not make sense to discard a large proportion of the germplasm that is high yielding, highly stable and adaptable but susceptible to GLS. The use of this germplasm will have to be monitored carefully within each breeding programme.

2.20 Assessment

Another factor which has not been quantified for GLS, is that of inter-plot interference (cryptic error) in small plot experiments (Vanderplank, 1963; Robinson, 1976; Zadoks and Schein, 1979; Robinson, 1987). As *C. zeae-maydis* is wind dispersed and produces vast quantities of spores on diseased plants, movement onto adjacent plants is rapid. In experiments where there are big differences is resistance or disease levels between the various entries or treatments, inter-plot interference is likely to be a significant factor. Research is needed to quantify this effect should adjustments need to be made to screening programmes or the composition of hybrid trial entries.

2.21 Fungicides

Fungicides are now used extensively in KwaZulu-Natal, the northern Eastern Cape and southern Mpumalanga to control GLS, particularly on farms where GLS-susceptible or partially GLS-resistant hybrids are planted. The basic factors governing fungicide usage and application have been established for local conditions (Ward *et al.*, 1993; Ward *et al.*, 1996; Ward and Nowell; 1997; Ward *et al.*, 1997a, 1997b, and 1997c; Chapter 9). Essentially the benzimidazole and triazole fungicides control GLS effectively. Duration between fungicide applications can be between 21 - 28 days. However, not all fungicides from these groups are equally effective. Protectant fungicides controlled GLS (Tables 9.4 and 9.5) but were not nearly as effective as the systemic fungicides from the above mentioned chemical groups.

It was decided that a dual active ingredient policy would be followed with commercial products to reduce the risks of resistance build-up in the fungus to any single active ingredient. This is in line with proposals for resistance strategies (Delp, 1980; Delp, 1984; Staub and Sozzi, 1984; Georgopoulos, 1986; Dekker, 1986; Wolfe and Barret, 1986; Delp, 1988; Scheinpflug, 1988; Wade, 1988). South African maize farmers are unlikely to follow a strict policy of voluntary fungicide rotation to reduce the risks of fungal resistance. For this reason it was decided that the safest option would be to ensure that dual active products only are marketed. Initially, benomyl and difenoconazole as single active products were registered for GLS control but these products were withdrawn as soon as combination products were registered.

Another factor that mitigates against resistance developing rapidly in *C. zeae-maydis* in South Africa is that only one or two applications are normally applied each season. In isolated cases,

three applications are undertaken. This will reduce the selection pressure on the fungus to develop resistance (Staub and Sozzi, 1984; Dekker, 1986; Wade, 1988). However, new products with different modes of action are tested every season. As yet none of the alternate products have been as effective as the above mentioned groups. This will continue to be an ongoing process until alternative products are found that effectively control GLS.

Of concern is the fact that no monitoring of *C. zeae-maydis* biotypes is taking place to check for resistance to the fungicides. A number of cases have been reported of fungicides not effectively controlling GLS, but to date these have all been application problems. Monitoring should be a co-operative effort between the Department of Agriculture and the suppliers of fungicides to control GLS on maize.

The GLS severity, likelihood of continued favourable conditions for GLS development, timing of application, choice of fungicide, frequency of application and the growth stage of the crop at application are all important when determining the economic viability of applying fungicides to control GLS on maize (Rivera-Canales, 1993; Martinson *et al.*, 1994; Wegulo, 1994; Jenco, 1995; Ward *et al.*, 1996; Ward and Nowell, 1997; Ward *et al.*, 1997a, 1997b, and 1997c). According to Ward *et al.* (1997b), the most practical way of determining the cost effectiveness of applying fungicide to control GLS, is by looking at the added profit from the process. This takes all factors into account and gives the net profit to the farmer which is likely from each hectare of maize. Under low inoculum pressure, the application of a third application is not justified financially. However, under high inoculum pressure, the highest added profit was from the treatments involving two or three fungicide applications. The increased profit from two to three fungicide applications was marginal and does not warrant the effort. The reduced efficacy of a fungicide treatment could be clearly seen when the disease was allowed to spread to the leaves at ear height before fungicide was applied. At this stage, a second fungicide application was not economically warranted due to the small increase in added profit.

The possible role of protectant fungicides alone and in combination with systemic fungicides has not been adequately researched. Initial tests have shown that the addition of protectant fungicides, such as chlorothalonil and mancozeb, to single or mixtures of systemic fungicides can be more effective in controlling GLS and at times, increasing grain yield (Nowell, unpublished). Their introduction into a spray programme would significantly reduce the fungicide costs. This may be particularly useful when GLS appears very early in a crop.

Chlorothalonil is also the only fungicide that is effective against *Phaeosphearia* sp. that can be problematic in some years. The use of protectants may have additional use in seed parent seed production fields, where some inbreds are particularly sensitive to several leaf diseases, including GLS.

Ward *et al.* (1997c) have determined the response of different hybrids to fungicide application. Such information is vital to farmers when trying to decide whether fungicide application is financially beneficial. This will also have to be done with the new hybrids when they are released. As GLS resistance levels increase, so the economic benefits of applying fungicides will decrease. Such information should also be available for hybrid parents for the use of seed companies in planning spray programmes. As this exercise is in progress, all diseases should be assessed and their effect on yield determined. *E. turcicum* (Nowell, unpublished) and *Puccinia sorghi* Schw. (Nowell and Rijkenberg, 1983) also cause yield losses in certain years in South Africa.

An additional benefit of fungicide application to control GLS is that other maize pathogens will be controlled at the same time. These diseases would include northern leaf blight, common rust, stalk rot and ear rot. Control of these pathogens could have significant economic benefits.

Only the copper-based fungicides have showed any degree of phytotoxicity (Chapter 9). This problem was consistent across products, locations and seasons. The data presented in this thesis did not show any interaction between fungicides and the environment. It is possible that certain triazole fungicides may have phytotoxic effects during periods of drought stress.

2.22 Fertility

Little research has been conducted with regards the effect of fertility on the incidence or severity of GLS. Smith (1989) found increased levels of GLS in response to increased nitrogen. This has been observed on some farms in the KwaZulu-Natal province. However, quantification of this response is currently taking place. At the same time, the effect of phosphate, potassium and soil acidity levels will be determined in the near future. It is important that fertility effects are determined under local conditions, as soils are very different in South Africa to those found in the U.S.A. This should be established under a number of divergent farming practices, including subsistence farming.

When conducting fertility trials, it is as important to get results from the low fertility plots as it is from the high fertility plots. Products such as cattle manure and chicken litter should be used to determine their effect on plant growth, *C. zeae-maydis* infection and subsequent GLS severity.

2.23 Effects on seed production

Although the effects on the profitability of commercial farmers is high (Chapter 8), the effect on a seed producer is greater as a number of additional factors are affected by GLS. Martinson *et al.* (1994) showed that detasseled maize had a higher incidence of GLS compared to male sterile inbreds. For this reason, it was suggested that fungicide applications should be used to counter this effect. This effect needs to be determined under local conditions. If GLS is a high sugar pathogen, then the removal of the tassel would reduce a carbohydrate sink and increase the level of carbohydrates in the upper canopy. An increase in GLS incidence or severity would be linked to the tassel size, or the amount of pollen produced (energy used) by the tassel, of a particular genotype. The effect on single cross parents may be greater as they usually have a proportionately larger tassel than inbred parents.

A study by Rivera-Canales (1993) showed that GLS not only affected the total grain yield of maize but also the saleable seed fraction and a lower weight for a specific number of seeds in seed production fields. Production costs are far higher than for commercial maize as the crop has to be detasseled by hand and management is far more intense. The value of the seed crop delivered to the seed producer is about 3 - 10 times greater in value than commercial maize, depending upon the type of maize seed produced. Any effect on yield or saleable fraction of seed has a major impact on profitability.

A reduction in the saleable seed fraction can have a major effect on both the seed producer and seed grower. In some cases, the growers are paid for the mass of saleable seed produced. A reduction in this fraction will affect profitability. For the seed producer, the reduced fraction will require having to produce a larger area to obtain the desired volume of seed. Where the grower is paid for the total seed delivered, the seed producer loses due to the reduced saleable fraction as a smaller proportion of the costs are recovered through seed sales to the farmer.

In South Africa, seed is sold per 25 or 50 kg bag of seed. The lighter grain mass means the

number of seeds per bag of seed will increase. This can be a significant loss to the seed producer, but of benefit to the farmer as he is able to plant a larger area for the same mass of seed purchased. This is one of the reasons South African seed producers are hoping to change to selling seed by the kernel number. In the USA a bag of maize seed normally contains 80 000 seeds.

Lighter seed implies the endosperm has not developed to its full potential. Table 9.6 showed that crude protein could be affected by GLS and the application of fungicides. This may affect germination, seedling vigour, plant establishment and the ability of the seed to store for a period of time (seed can be stored up to three years). The reduced seed health could predispose the seedling to agrochemical toxicity problems. No information is available on these issues. With the large effect GLS can have on grain yield and quality, it is desirable that these issues be tested locally. All variables need to be monitored to determine the effects of GLS on seed health.

The susceptibility of hybrid parents to GLS will largely influence the decision as to where to produce the hybrid. This would mean a change in the logistics associated with certain hybrids and may limit the volume of seed which can be produced in areas that GLS is problematic. This impacts on the farmers who rely on producing seed for a living. Fortunately, maize hybrids in South Africa seldom have pure inbred lines as the parents as single cross hybrids are presently not cost effective for the farmer. However, the parent seed of the parents is produced on inbreds because the cost of this operation has increased dramatically through the use of fungicides to control GLS. The costs of harvesting the seed crop have also increased as seed is essentially hand harvested in South Africa and increased lodging has slowed down the process. The increased costs of these operations has ultimately been borne by the commercial farmer.

2.24 Weeds

Spink and Lipps (1987) mentioned that weed control is important to increase airflow within the canopy and thereby reducing favourable conditions for infection. This is particularly relevant to Africa, as subsistence farmers seldom practice adequate weed control. The "weed effect" would be greatest in the more humid and warm environments. Weeds will also compete for nutrients and affect plant health. A small effect like this could be important for subsistence farmers.

2.25 Effect on other pathogens

No literature is available regarding the interaction of *C. zeae-maydis* and other pathogens. The fact that GLS has received so much attention due to its effect of the grain yield, does not mean that other previously problematic diseases have reduced in importance. There are still *E. turcicum* and *P. sorghi* epidemics in the eastern maize production regions of South Africa. Those farmers that are applying fungicides will be controlling a range of fungal pathogens, although not all equally well. The interaction of these pathogens with GLS is not known and needs investigation. Since GLS causes crops to dry off earlier than normally expected, a reduction in ear rot has been observed.

2.26 IPM in localised areas / farms

Prior to GLS arriving in South Africa, maize was a crop that only required monitoring for weeds and stalkborer once it had been planted and established. In areas where GLS is endemic, the management level required of maize farmers has increased dramatically. At present there are no agronomic practices that can be changed that will eliminate GLS or reduce its effects to sub-economic levels. The farmer now has to design an integrated programme that will reduce the risks of GLS at all stages. This starts with land preparation, planning which crops to plant, selecting GLS-resistant hybrids that are suitable for the farm and the objectives of growing maize, monitoring the field for GLS and subsequent development of the disease, and ensuring the correct application and usage of fungicides when necessary. Once hybrids become available that will reduce GLS to negligible levels, management of a maize crop will again become a relatively simple process.

The requirement of increased management from maize farmers has also resulted in these farmers becoming more aware of other details within the crop. There has been a large increase in queries about other diseases and their effects on the yield and quality of the crop. Many other previously unnoticed agronomic problems are now being observed and corrected. It has also ensured considerable improved communication between the farmers and their input suppliers. Farmers' days and farmers' work groups have helped considerably in finding and promoting solutions to the GLS problem. From this point of view, the occurrence of GLS has had a beneficial effect on maize farming.

2.27 Prediction model / forecasting

Computer based prediction systems have been discussed and some are in the process of being tested in KwaZulu-Natal (Ward and Berry[38], pers. comm.). A prototype model was run during 1995/96 and the model was able to closely predict the onset of GLS in a number of areas. However, the predictions are not equally accurate for all regions. A limiting factor is that the exact temperature requirements of the pathogen under local conditions have not been established, particularly the effect of temperatures lower than 20°C. This system could be used to warn farmers when to start monitoring their crops closely. For maximum benefit when planning a fungicide programme, each farm would have to have their own weather monitoring system, and hybrid resistance would have to be built into the model. Considerable work is still needed before this type of model could be used for anything more than an early warning system for the start of a GLS epidemic or warning of favourable conditions for infection.

2.28 Communal farmers

Commercial or large scale farmers in South Africa have overcome the effects of GLS in the short term through the use of fungicides to control GLS (Ward *et al.*, 1993; Ward and Nowell, 1997). This is economically viable for commercial farmers (Ward *et al.*, 1996), but is not an option for subsistence farmers who do not have the capital, and often the knowledge, to purchase the fungicides or equipment to apply the chemicals. This would apply to the majority of farmers in South Africa and in the rest of Africa. For the subsistence farmers, enhanced levels of GLS resistance with improved cultural practices or alternate crops are the only solutions. Farmers will need highly GLS-resistant hybrids to successfully and permanently control the disease.

Subsistence farmer can afford minimal inputs, if any at all, and as a result the crops usually suffer from nutrient deficiencies. From the authors observations in the subsistence and small scale farmers' fields in Cameroon, Kenya, South Africa and Zimbabwe (and the little information that is available on fertility), indications are that the less healthy a crop, the less likely it is to be infected by *C. zeae-maydis* and that disease development will be slow. It will be the crops of the more progressive subsistence farmers that improve plant health that will have

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the highest incidence of GLS.

Another fact is that if ears are removed from a healthy plant, GLS increases rapidly on this plant compared to those plants where the remains intact on the plant. This is apparent in fields harvested for fresh maize consumption. GLS is a high sugar disease and will be more severe in healthy plants than plants that are stressed or have limiting growth factors. This is circumstantial evidence suggesting that subsistence farmers in general should not have as severe GLS epidemics as farmers that have high inputs and a healthy crop.

The effect of a GLS epidemic is felt more by subsistence farmers as not only is the grain yield important for their existence, but the remaining diseased plant material is fed to animals. There will be less plant material and the quality of this material will be lower than that from a healthy crop. An advantage of feeding the plants to animals after harvesting the grain, is the reduced GLS inoculum level the following season. However, it needs to be determined whether *C. zeae-maydis* conidia are viable after passage through animals and if they can then still infect maize.

The breakdown of VR would have large implications for commercial farmers but alternate strategies can be introduced rapidly and the effect of the disease on the crop can be reduced rapidly. The survival of subsistence farmers' depends on the success of their maize crops and they cannot afford a crop failure. Nor can these farmers afford fungicides or the equipment that is required to apply to them. Further, change takes place very slowly, especially to new cultivars even if they can obtain seed. From a food security point of view, VR should not be introduced into areas were people are absolutely reliant on their own maize crops to feed their families. With a disease as severe as GLS, famine could be the result of a major breakdown of VR.

Most of the open pollinated cultivars currently grown by small scale farmers in South Africa are highly susceptible to GLS (Nowell, unpublished). Should GLS become a problem in specific cases, more resistant hybrids are readily available locally. However, the farmers will have to be educated into buying fresh seed each year, due to the inbreeding depression experienced when using F2 seed. Many small scale farmers have started changing to the use of higher yielding and stable hybrids. As these farmers plant almost exclusively white-grained hybrids, the level of GLS resistance in usually already high.

Many small scale farmers and subsistence farmers practice intercropping of a variety of crop species. The effects of intercropping maize and legumes could result in healthier maize plants due to the increased nitrogen made available by the legumes, which will probably result in GLS having a larger effect on the maize crop. Intercropping trials throughout KwaZulu-Natal should be monitored for this effect.

3 MANAGEMENT STRATEGIES

The recent epidemics of GLS and ear rot on maize in South Africa have had a large impact upon the maize industry and resulted in changes in long term planning of maize research and breeding programmes. It has also improved co-operation between researchers, particularly plant pathologists, and extension personnel working in the maize industry. This has allowed for the rapid response to unexpected and urgent maize disease problems, with a minimum of restructuring and financial input.

Ideally, there should be a clear strategy to combat new maize diseases or epidemics. Response to such threats should be structured and co-ordinated. This would allow the optimum use of the expertise and facilities available in South Africa to counter any maize disease threat. Such a response would ensure that the necessary research is conducted in the shortest time possible. Farmers would benefit by getting reliable disease control information as soon as practically possible. Had such a policy being in place in the mid-1980s, the extent and effect of the ear rot epidemics could have been substantially reduced. However, such a strategy did not exist in South Africa and there is still a lack of clear overall policy regarding the monitoring of maize diseases and the implementation of a disease control strategy. There are a number of reasons for this:

i) There are few plant pathologists working solely on maize
ii) Most of the government or quasi-government research institutes where maize plant pathologists are based in areas of low maize disease risk, making pathology research difficult or limited to a narrow range of diseases
iii) Other researchers working on maize diseases are scattered around the country, seldom being based at sites where disease problems are endemic and important
iv) Policy cannot be made by any one person or body due to the many stake holders involved in the maize research
v) In the past, the internal politics of the maize industry has played a significant role in

leading to conflicting agendas and poor overall planning

vi) Due to the fragmentary nature of research in the maize industry, funding for maize pathology research is difficult to co-ordinate and often difficult to obtain.

Following deregulation, resulting in the South African Maize Board playing a smaller role in determining industry priorities and policy in future, it may now be possible to introduce a more structured and planned national maize pathology strategy. However, considerably closer co-operation is needed between commercial organisations within the maize industry, the various Agricultural Research Council Institutes and the Universities in South Africa. A problem will be to find the organisation / person with maize pathology experience that is capable of fulfilling this role.

If the maize industry is not capable of becoming more organised, maize disease epidemics will continue to catch the maize industry "unexpectedly" in years to come. As an example, maize eyespot (caused by *Aureobasidium zeae* [Narita & Hiratsuka] Dingley) is already beginning to concern farmers and some maize pathologists (Flett and Nowell, 1995), yet little is being done to combat or understand this disease. Yield losses have been substantial in localised areas in the Midlands of KwaZulu-Natal and the disease is known to be severe in localised areas of the eastern Free State and Mpumalanga provinces of South Africa.

The epidemiology of GLS and *Stenocarpella* ear rot are dissimilar in many aspects but some factors are common. Both pathogens are essentially reliant on maize debris for their continued survival and the production of inoculum to incite disease in the subsequent crop. This means that crop residues should be an important control measure. Resistance is an effective means of controlling these pathogens, although stress can predispose maize to ear rot infection. Fungicides can effectively control both diseases but ear rot could not be controlled by fungicides applied by aircraft (Farwell, unpublished). Low plant densities appear to enhance both diseases, but further research is needed to clarify this point. Irrigation has been shown to be an important factor in increasing the incidence and severity of both these diseases in maize.

The major difference is that GLS is a polycyclic pathogen that is easily wind dispersed over considerable distances, whereas *Stenocarpella* ear rot is a mono-cyclic pathogen, except for the leaf blight phase of *S. macrospora*, that is primarily splash dispersed. Conidia of the *Stenocarpella* species are primarily splash dispersed, which means crop rotation and tillage has a large effect in controlling the disease. In contrast, *C. zeae-maydis* is wind dispersed and crop

rotation and tillage have a minimal impact in controlling GLS.

The benefits of conservation tillage under South Africa's the variable climate are enormous. For this reason strategies have to be developed that ensure conservation tillage can be practised without major disease problems and consequent economic losses. The long-term objective should be to increase inoculum pressure during selection in maize breeding programmes and testing of hybrids, to ensure hybrids of improved disease resistance are marketed in future. In the interim, supplementary/alternative strategies, such as rotation, plant health and the judicious use of fungicides, should be promoted.

Since GLS became epidemic in KwaZulu-Natal, there have not been any *Stenocarpella* ear rot epidemics. GLS makes the plants mature sooner than would normally be expected. This may limit the ability of *Stenocarpella* species to develop fully in the ears. Research is needed to determine whether this effect interaction occurs or is merely an artifact of environmental conditions.

The use of ethographs is a very useful way of understanding the disease overall and can be used effectively to make comparisons between pathogens. Their use as a management tool should not be underestimated as the weaknesses in the disease cycle are easy to identify as intervention points to improve disease control. Ethographs can also be used to identify areas where research is needed for a better understanding of the pathogen.

The education of farmers and personnel in the maize industry with regard to maize diseases should be an ongoing process. In the past, little has been done to adequately inform people involved in the maize industry of the details of a given problem. Education is needed to correct erroneous perceptions, introduce new concepts and improve existing knowledge on maize diseases. This should not be a one way process. Considerable feedback from people in the field can be obtained while the education process is in progress. There is a need for overall co-ordination of this education process. It would be desirable to emphasise plant health as a whole, rather than concentrating on problems in isolation. Specific programmes need to be devised for the end user; e.g., small scale farmers cannot be treated in the same way as large commercial farmers, and researchers have different priorities compared to farmers. When training people, maximum interaction can be obtained while discussing the programme in the field with examples. This allows for feedback from the audience to the trainer and practical problems can

be discussed in the fields. The education process should make use of all types of facilities and media available, although direct interaction would be the best method. As an example, many of the people involved in the maize industry are of the opinion that resistance means that the host will not be infected or colonised by the pathogen, and quantitative infection is a viewed as a degree of susceptibility. However, pseudo-immunity is usually high level qualitative resistance, a form of VR, which has inherent stability problems. Quantitative resistance is a desirable trait as it is usually more stable in nature (Vanderplank, 1984; Robinson, 1987). It is the level of resistance under a given inoculum pressure that is important. This perception needs to be changed so that farmers and researchers can understand the mechanisms and utilise resistance to its maximum, and not only opt for pseudo-immunity. Vertical resistance should in fact be avoided, particularly when the cultivar with VR is marketed to subsistence farmers.

A frustrating aspect of current maize pathology research in South Africa is that the public maize pathologists are based in an area of low disease risk, especially leaf diseases. It is vital that pathology research be conducted in areas of disease occurrence, so that the results are applicable to the problem. This also cuts down considerably on the costs of transport to areas of high inoculum pressure. Being based in areas of high disease risk, trends is diseases and problems can be seen long before they become problematic to the maize industry as a whole. However, this would require a shift from Potchefstroom in the North West province to Cedara in KwaZulu-Natal of staff, equipment and budgets.

The seed companies should be a partners in pathological research and disease problem solving, and not be expected to undertake their own basic pathology research. Their primary objective should be to provide solutions to disease problems through new and improved hybrids or varieties. They can also be used to educate farmers and the relevant agricultural personnel.

Funding or partial funding of research projects of common interest could come from the maize industry. A problem is that many of the people involved in the maize industry still believe that the government should provide the finance for this type of research. A change in attitude in the short term is unlikely.

Biotechnology is likely to play an important role in agriculture, and in maize in particular, in the future. The ability to introduce exotic genes into maize and the use of marker-assisted breeding techniques are just two of the techniques that could be of enormous benefit to maize

breeding and maize production. After many years of basic and applied research, maize hybrids developed with the help of biotechnology are being commercialised for the first time. This has given rise to many regulatory and marketing problems that now need to be finalised before maximum benefits can be obtained from these products. Many new forms of disease resistance are nearing commercialisation to a range of pathogens (Walker[39], pers. comm.; Ziegler[40], pers. comm.).

However, the practice of introducing single genes for resistance in to maize could give rise to disease epidemics in future. These disease resistance genes that are likely to confer VR to maize. Vertical resistance will not be a stable form of resistance (Vanderplank, 1984; Robinson, 1987) and will give rise to epidemics in future when the pathogen's virulence gene matches the resistance gene. Management of this resistance will be complex as new forms of these genes will have to be continuously available. This is apparently the strategy being followed for the Bt-gene that is being released in maize for insect resistance (Walker, pers. comm.). Management of VR, introduced by the transformation of maize, will have to be managed carefully. Hopefully, as transformation techniques improve, and other techniques become available, HR genes will replace the use of VR genes.

The use of quantitative trait loci (QTLs) in breeding programmes gives maize breeders a valuable tool to breed more complex disease resistance, and hopefully HR, into maize. The first tentative steps in this direction are already in progress for GLS with the research of Bubeck *et al.* (1993) and Saghai Maroof *et al.* (1996). Hopefully this technology will be developed further and there are plans to use it locally in the near future. Due to the distribution and severity of GLS in Africa, HR would be the ideal form of resistance to GLS, particularly as small scale / subsistence farmers will be the main recipients of the GLS-resistant maize.

Grey leaf spot was a new disease to occur in South Africa that expanded in distribution and severity rapidly once it had established itself. *C. zeae-maydis* is not seed-borne or seed transmitted (McGee, 1988) and the use of phytosanitary regulations would not have helped to keep the pathogen out of the country or limited its spread in the country. This epidemic has

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made South Africans aware of the risks of new diseases if the local environmental conditions are suitable for the pathogen. In the past South African phytosanitary regulations have not always been based on sound pathological principles and thorough risk analysis. This now needs to be reviewed for all crops, particularly on maize due to its economic importance to the agricultural sector of the South African economy.

All phytosanitary regulations are based on the diseases that are known to occur in the country. However, there are a number of maize diseases that have not been officially reported or identified in South Africa. Most of these pathogens are in the KwaZulu-Natal and Mpumalanga provinces and this needs to undertaken as a matter of urgency. Other crops are in an equally poor state with respect to the identification of diseases already occurring in South Africa.

There is considerable political pressure at present to develop a Southern Africa trade zone. Should this materialise, it will have significant implications for management of plant diseases in the region. At present each country is autonomous with respect to phytosanitary matters, but the creation of the Southern African trade zone would necessitate considerable co-operation between the relevant authorities. As the staple crop in the region, maize would be the major crop. An overall strategy governing phytosanitary regulations and disease control, particularly of economically important diseases, would have to be negotiated. Hopefully, the experience gleaned over the past few years (and the next few seasons) on downy mildew on sunflower, GLS on maize and frogeye on soybeans will form the framework for future co-operation.

The *Stenocarpella* spp. and *C. zeae-maydis* are pathogens that are part of the overall maize pathogen complex. The fungi causing ear rot are particularly complex (Koehler, 1959 and 1960) but each pathogen should not be viewed to the exclusion of the others. Management of maize disease resistance is vital to minimise the impact of disease in maize production. When releasing new germplasm into the market it is important to ensure that this material is not highly susceptible to a pathogen that is usually of minor importance. If this should take place, a minor disease could establish itself and the inoculum pressure would increase substantially with the increased planting of this susceptible hybrid. In this way man can create his own epidemics. It is suspected that this is what is currently happening with eyespot of maize in the KwaZulu-Natal and Mpumalanga provinces. A susceptible hybrid is now widely grown and farmers are experiencing economic losses under specific environmental conditions.

The interaction of host, pathogen, environment and man is a dynamic process that needs careful and close monitoring. Any changes in this balance need to be noted quickly and strategies to combat any imbalances developed. If these changes are not observed timeously and countered, damaging epidemics can and will occur periodically. Hopefully, people in the maize industry in South Africa have learnt a number of lessons from the ear rot and GLS epidemics in the recent past, and the necessary adjustments will be made so the effects of maize diseases in future will be minimised.

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