

MAIZE ROOT ROT IN SOUTH AFRICA

by

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## DECLARATION

I hereby declare that this dissertation is the result of my own investigation and has not been submitted to any other university.

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## ABSTRACT

Numerous fungal species are known to infect maize roots and eventually cause rot. The spectrum of fungi differs over localities as well as their relative frequencies. Fungi isolated from discoloured root tissue and root tissue without visible discolouration were classified as root pathogens and root colonizers, based on their isolation frequency from the respective samples. *Exserohilum pedicellatum*, *Macrophomina phaseolina* and *Fusarium oxysporum* were classified as the major root pathogens and *Phoma* spp., *Curvularia* spp. and *F. chlamydosporum* as root colonizers. Fungi classified as root pathogens tended to occur early in the growing season in juvenile tissue as opposed to root colonizers which occurred later. Treatments in an existing long-term water stress trial included no stress (irrigation), normal rainfall, stress until flowering, stress after flowering until mid grain-fill, stress after flowering and total stress. Root pathogens were isolated at higher frequencies in the no stress and normal rainfall treatments than in the total stress treatment. A positive linear relationship between the water stress index and the isolation frequency of *M. phaseolina* was obtained. Negative, non-linear relationships were, however, recorded for *E. pedicellatum*, *F. oxysporum* and *F. moniliforme*. The effect of tillage practices on disease severity was carried out in field trials at two localities. Tillage practices applied included rip, plough, chisel plough and no-till treatments. Significant differences between isolation frequencies and tillage treatments were recorded for *F. oxysporum* at Bloekomspruit and *Trichoderma* spp., *Alternaria* spp. and *M. phaseolina* at Mmabatho. Differences in isolation frequency of fungi involved in maize root rot, were determined in a crop rotation system where maize was rotated with soybeans, sunflowers and groundnuts. Crop rotation had a significant effect on the isolation frequency of *Thielavia terricola*, *E. pedicellatum*, *F. moniliforme* and *F.*

*graminearum*. The effect of crop rotation, however, seems to be complex since fungi were affected differently in the various treatments.

## CHAPTER 1

### GENERAL INTRODUCTION

Maize root rot occurs worldwide and has been extensively studied in Canada (Whitney & Mortimore, 1957; 1961; 1962), the USA (Shepherd, Hall & Pendery, 1962; Shepherd, Butler & Hall 1967; Sumner & Bell 1982; 1986), the Netherlands (Scholte & s'Jacob, 1983; Scholte, 1987) and South Africa (Du Toit, 1968; 1969; Chambers 1987a;b; Deacon & Scott, 1983). Although maize root rot and its cause has not been reported in the South African literature until the late 1960's, Du Toit (1968) suggested that root rot may have been present since maize was first cultivated in this country.

Maize root rot is frequently associated with stalk rot (Whitney & Mortimore 1957; Williams & Schmitthenner, 1963; Sumner, 1968; Dodd, 1979). Whitney & Mortimore (1957), emphasized that maize plants may have totally diseased roots without having stalk rot, but that stalk rot is always accompanied by root rot. Sumner & Bell (1982) recorded the absence of stalk rot on maize plants with severe root rot. Although most fungal pathogens of maize stalks would also attack roots, some species seem to be restricted to either stalks or roots (Ullstrup, 1977). Chambers (1987a), however, found no correlation between incidence of root and stalk rot under South African conditions.

Concerning yield loss due to root rot, very little evidence of a direct association is available (Williams & Schmitthenner, 1963; Le Roux, 1977; Sumner & Bell, 1982; 1986; Scholte 1987; Chambers, 1987b; Lipps, 1988; Sumner, Gascho, Johnson, Hook & Threadgill, 1990).



Symptoms associated with maize root rot, especially above-ground, can be very deceptive. Maize plants subjected to root rot are characterized by poor stands and uneven growth, while older plants are stunted and chlorotic (Richardson, 1942). In such cases, root systems are often rotted to such an extent that only a few secondary roots are still functional, eventually resulting in lodging of plants. Lodging of maize plants caused by root rot occurs at the soil surface and not between the fourth and fifth internode as in the case of stalk rot. Sumner & Bell (1982) found that diseased plants were occasionally stunted and chlorotic, but plants with severe crown and lateral root rot could not be distinguished above-ground from plants with well developed root systems, unless the plants were lodged. The incidence of root rot is usually indicated as root discolouration and poor root development. These criteria, however, are suspicious since various environmental and mechanical factors may also contribute to root discolouration and poor development. In the present study, maize roots were classified either as visibly discoloured root tissue or root tissue without visible discolouration i.e. clean root tissue. Fungal species were isolated from these root tissue samples and based on their isolation frequencies, were regarded either as pathogens or colonizers.

16 The organisms involved in the maize root rot disease complex may vary considerably between localities. Ho & Melhus (1940) concluded that the majority of fungi occurring in the root rot complex, are soil inhabiting fungi that infect maize roots under various environmental conditions conducive to their optimal needs. These environmental conditions include various factors, such as temperature, water and the nutrient status and physical properties of the soil (Manns & Phillips, 1924; Thayer & Williams, 1960; Möhr & Le Roux, 1977). Maize root rot was found to be more severe during times of plant stress, especially during drought conditions (Du Toit, 1968; Chambers, 1987a), but also in conditions where

soil water was excessive (Sumner, 1968; Sumner & Bell, 1982). Roots of maize plants are exposed to fungal populations for much of the season and are prone to invasion by soilborne fungal pathogens (Dodd, 1980). Research results to date are inconclusive on the most important pathogens in the complex (Miller, 1964; Palmer & Kommedahl, 1969; Deacon & Scott, 1983). Although numerous soilborne fungi are common to different soils, the predominant pathogenic fungi may differ between localities.

Ho & Melhus (1940) grouped root rot fungi according to their disease-inducing capacity, while Young & Kucharek (1977) illustrated that different fungi may infect maize roots at different plant-growth stages. Fungi commonly isolated from maize roots, which are regarded as severe root rot pathogens, include *Fusarium* spp. (Rao, Schmitthenner, Caldwell & Ellet, 1978; Miller, 1964), *Pythium* spp. (Rao *et al.*, 1978; Hellinga, Bouwman, Scholte & s'Jacob, 1983), *Rhizoctonia* spp. (Sumner & Bell, 1982; 1986) and *Exserohilum pedicellatum* (Henry) Leonard & Suggs (Shepherd *et al.* 1967; Du Toit, 1968; Chambers 1987a; b). Recent research focused on both specific organisms and the root rot pathogen complex as a whole (Blanquet, Van Schingen, Foucart, Maraite & Ledent, 1990; Leslie, Pearson, Nelson & Toussoun, 1990). Various discrepancies regarding the pathogenicity of fungi involved in maize root rot have not yet been addressed satisfactorily, indicating the complexity of this disease.

The effective control of maize root rot is difficult to achieve, mainly because of the wide spectra of fungi associated with this disease. Chemical control is often not economically justifiable. Alternative control measures such as cultural practices and host plant resistance to the disease may offer a long-term solution to the problem. Yield reduction as a result of

maize crown and brace root rot may be greater if root growth is restricted by soil compaction. Accordingly, the incidence of *Fusarium* spp. in maize sub-crown mesocotyls and crowns tended to be higher in plants from no-till treatments than from ploughed treatments (Lipps & Deep, 1991). Continuous reduced tillage and monoculture of crops have been shown to increase the amount of inoculum for many diseases (Boosalis & Doupnik, 1976; Sumner, Doupnik (Jr.) & Boosalis, 1981). In South Africa, maize is traditionally produced under a monoculture system. Channon & Farina (1991) found that crop rotational trials with soybeans in Natal resulted in dramatic maize yield increases, which could not be ascribed to N-nutrition or stalk rot incidence. Soil-related factors differ considerably under various tillage and crop rotation practices and justify research on their influence in maize root rot incidence. Maize roots were sampled in different tillage practices and crop rotation systems and the isolation frequency of fungi associated with maize roots were determined. This was done to conclude whether these cultural practices affect the incidence and occurrence of maize root fungi.

AB Apart from studies to identify the maize root rot pathogens, the full impact of this disease, especially on yield loss and conditions under which the disease prevail, warrants more research. Contradicting results regarding the pathogenicity of fungi involved in maize root rot emphasise the complexity of this disease. The primary purpose of the present investigation was to obtain basic information on the effect of water stress, tillage practices and crop rotation systems on the incidence and spectra of fungi associated with maize root rot. This information is deemed essential to progress with the composition of an integrated disease control programme and the prevention of crop losses due to this disease.

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## CHAPTER 2

### FUNGI ASSOCIATED WITH MAIZE ROOTS IN SOUTH AFRICA

#### ABSTRACT

Reports on the etiology of maize root rot are contradictory. A spectrum of fungi have been reported, but the relative importance of each fungal species and its role in the disease complex have not yet been elucidated. The spectrum of fungi associated with maize root rot may differ over localities as well as in frequency. This study was conducted to determine the isolation frequency of fungi from maize roots. Fungi were isolated at different plant growth stages and from discoloured and clean root tissue samples. Fungi isolated were classified into root pathogens and root colonizers based on their isolation frequency in discoloured and clean root samples. Fungi classified as pathogens included *Exserohilum pedicellatum*, *Macrophomina phaseolina* and *Fusarium oxysporum*, whereas *Phoma* spp., *Curvularia* spp. and *F. chlamydosporum* were classified as root colonizers. Root pathogens have the ability to cause discolouration when they occur in maize roots whereas, the incidence of colonizers did not differ significantly between discoloured and clean root samples. Fungi classified as root pathogens tended to occur early in the season in juvenile tissue as opposed to root colonizers which occurred later in the season.

#### INTRODUCTION

Since the earliest published records (Manns & Phillips, 1924), researchers have attempted to isolate and identify the causal organisms associated with maize (*Zea mays* L.) root rot.

The relative importance of various fungi in the root rot complex has not been elucidated and research results are contradictory. Indications are that several fungi and even bacteria are involved, the spectrum depending to a large extent on environmental conditions (Mortimore & Ward, 1964). Chambers (1987a) stated that fungi infecting maize roots are facultative parasites occurring in the soil and under the seed coat. Variation of fungal colonization within plant roots is high and differ in frequency from that found in soil or debris. It is therefore, often difficult to discern whether the fungus is the primary disease-causing agent, a secondary invader or an endophyte (Leslie, Pearson, Nelson & Toussoun, 1990). Fungi in the root rot complex have been regarded as saprophytes and can be classified as "minor pathogens" (*sensu* Salt, 1979), since they are widely distributed in cultivated soil and have a wide host range. Although some *Fusarium*, *Rhizoctonia*, *Pythium* and *Exserohilum* spp. can be classified as "important pathogens" in the root rot complex (Shepherd, Hall & Pendery, 1962; Shepherd, Butler & Hall, 1967; Rao, Schmitthenner, Caldwell, & Ellet, 1978; Chambers, 1987b), some "minor pathogens" such as *Trichoderma*, *Curvularia*, *Penicillium* and *Altenaria* spp. may also play an active role in the root rot complex (Ho & Melhus, 1940; Young & Kucharek, 1977). Ho & Melhus (1940) grouped root rot fungi according to their disease-inducing capacity, whereas Young & Kucharek (1977) illustrated that different fungi infect maize roots at different plant-growth stages and identified five fungal communities.

The symptoms associated with maize root rot, especially above-ground, can be very deceptive. Stunting and lodging of maize plants are the most common. Lodging of maize varies from season to season, the extent of lodging depending on environmental conditions (Du Toit, 1968; Thompson, 1972). Root discolouration and development is frequently

associated with maize root rot (Hornby & Ullstrup, 1967; Shepherd, *et al.*, 1967; Sumner & Bell, 1982), although the accuracy of these criteria are questionable. This study was initiated to determine the incidence of root-infecting fungi in discoloured root tissue as opposed to root tissue visually free from symptoms.

## MATERIALS AND METHODS

This chapter is a synopsis of the following chapters (Chapters 3-5) and is presented to identify the important fungi as a preliminary chapter to the thesis.

Maize field trials were surveyed at four localities during the 1992/93 and 1993/94 seasons. Trials included a water stress trial at Potchefstroom (Chapter 3), tillage trials at Bloekomspruit and Mmabatho (Chapter 4) and a crop-rotation trial at Viljoenskroon (Chapter 5). Potchefstroom, Bloekomspruit and Mmabatho trials were laid out on Hutton soil types with clay contents of 35%, 23% and 11%, respectively. The trial at Viljoenskroon was laid out on a Clovelly soil type with a clay content of 12%. The maize cultivars PAN5206, PAN6549, PAN6528, and PAN473 were planted at the localities, respectively.

Trials were laid out according to a randomized block design with three replicates. Plants were maintained until yield was determined, applying weed and insect control as required. A total of 5670 root systems were investigated during the two seasons.

Sampling commenced four weeks after planting and was done at fortnightly intervals until tasselling. Maize plants were randomly dug out, endeavouring to maintain as much as

possible of the root system. Plant lengths (crown to tip of apical leaf) and dry mass (drying at 60°C until constant mass was obtained) of aerial plant parts were determined and visual assessments of the percentage root rot were carried out only during the 1992/93 season. Yield was determined from remaining plants at plant maturity.

Roots recovered from each sample were thoroughly washed in water to facilitate the removal of all soil and debris. Visible discolouration (DR = discoloured roots) were dissected from roots, pooled, cut into 3 mm segments and sterilised in 3.5% NaOCl for 1 min. After being rinsed twice in sterile distilled water, root segments were plated out onto Potato Dextrose Agar, Malt Extract Agar and Potato Carrot Agar. The number of segments plated out ranged from 600 to 1050 for each sampling date. Cultures were incubated at 25°C for 3 days after which fungi isolated from maize roots were identified and quantified. Isolated *Fusarium* spp. were plated out on Carnation Leaf Agar for identification. Root segments with no visible discolouration (CR = clean roots) were treated similarly and in equal numbers. Differences in isolation frequency between DR and CR root samples were used to distinguish between fungi that may be regarded as root pathogens and root colonizers.

Data were analyzed using analysis of variance. Scheffé's LSD was used to determine differences between the incidence of fungi isolated from roots at different localities and between DR and CR root samples. The factorial of the replications was not crossed, but nested within the localities.

## RESULTS AND DISCUSSION

The spectrum of fungi isolated from maize roots in this study (Table 1) corresponds with the spectra of fungi most commonly isolated from maize roots (Shepherd *et al.*, 1962; Du Toit, 1968, 1969; Futrell & Kilgore, 1969; Chambers, 1987a). *Phoma* spp., *Trichoderma* spp., *Macrophomina phaseolina* (Tassi) Goid, *Exserohilum pedicellatum* (Henry) Leonard & Suggs, *Fusarium oxysporum* Schlecht. emend. Snyder & Hans, *F. chlamydosporum* Wollenw. and *F. equiseti* (Corda) Sacc. were isolated at highest frequencies. Some isolates i.e. *Alternaria* spp., *Curvularia* spp., *Thielavia terricola* (Gilman & Abbot) Emmons, *F. moniliforme* Sheldon, *F. culmorum* (Smith) Sacc., *F. graminearum* Schwabe, *F. compactum* (Wollenw.) Gordon and *F. sambucinum* Fuckel were isolated at specific localities at relatively low frequencies. Although *F. moniliforme* and *F. graminearum* are considered to be important pathogens in the maize root rot complex (Miller, 1964; Rao *et al.*, 1978), these fungi were relatively infrequently observed in this study.

The isolation frequency of *F. moniliforme* and *F. graminearum* did not differ significantly between DR and CR root samples, suggesting that these fungi are not primary lesion-causing pathogens on maize roots under local conditions. Chambers (1987a) found isolates of *F. moniliforme* to be weakly pathogenic on maize in South Africa, while Hornby & Ullstrup (1967) only occasionally isolated *F. graminearum* from maize roots in the USA. Those fungi that tended to occur at relatively low incidence and did not differ significantly in frequency between DR and CR root samples should be regarded as root colonizers rather than pathogens.

However, the isolation frequency of *E. pedicellatum* was significantly ( $P < 0.05$ ) higher in the DR than in the CR root samples (Fig. 1), suggesting that this fungus tends to cause discoloured roots where it occurs in maize roots. *Trichoderma* spp, *M. phaseolina*, *F. oxysporum* and *F. equiseti* showed similar tendencies and may, along with *E. pedicellatum*, be regarded as root pathogens rather than root colonizers. *E. pedicellatum* was found to be pathogenic on roots of maize and sorghum, causing severe root discolouration (Shepherd, *et al.*, 1962; Shepherd, *et al.*, 1967; Du Toit, 1968; Chambers, 1987b). *T. lignorum* Harz can be slightly to non-pathogenic (Ho & Melhus, 1940) and *Trichoderma* spp. were most commonly isolated during the early stages of plant development in the USA (Sumner, Gasho, Johnson, Hook & Threadgill, 1990) and South Africa (Chambers, 1987b). Although Young & Kucharek (1977) isolated *M. phaseolina* on maize roots, it is more commonly associated with stalk rot in maize and sorghum and root rot of sorghum, occurring most frequently under drought stress conditions (Edmunds, 1964; Mughogho & Pande, 1984; Pande, Mughogho & Kurunakar, 1990). *F. oxysporum* is commonly isolated from maize roots (Hornby & Ullstrup, 1967; Scott, 1982), although contradictory views are held regarding its pathogenicity. Palmer & Kommedahl (1969) found that *F. oxysporum* primarily causes wilting of plants in many crops, but is not a pathogen of maize roots except in sterile soil with a high inoculum potential. However, *F. oxysporum* was numerically dominant in rotted maize roots in the USA throughout the season (Hornby & Ullstrup, 1967). Sorghum roots are also frequently infected by *F. oxysporum*, *F. equiseti* and *F. solani* (Reed, Partridge & Nordquist, 1983; Odvody & Dunkle, 1984).

Some fungi isolated at the four different localities (Fig. 1) showed no differences in incidence between DR and CR root samples. Chambers (1987a) concluded that the ability of fungal



species to cause maize root rot is an isolate rather than a species attribute. Pathogenicity tests should therefore, concentrate to determine the variation of pathogenicity within a species.

It is assumed that colonization of maize roots by root pathogens occurs primarily late in the season, since root rot is associated with lodging and senescence (Mortimore & Ward, 1964; Thompson, 1972; Dodd, 1980). This, however, was not the case with the fungi classified as root rot pathogens in this study. *E. pedicellatum*, for example, occurred early in the season in juvenile tissue and declined as tissue matured (Fig. 1). Greater colonization of actively growing, juvenile tissue compared with older, senescent tissue questions the theory that root rot is a disease of senescing tissue. The progressive decline in the *E. pedicellatum* population suggests that the organism is unable to compete saprophytically towards the end of the season, emphasizing its pathogenic ability. Fungi classified as root colonizers eg. *Phoma* spp., *Curvularia* spp. and *F. chlamydosporum*, occurred late in the season when plants were maturing, suggesting an inability of these fungi to colonize actively growing tissue (Fig. 1).

Although correlations with root discolouration and plant height and dry shoot mass have been recorded (Hellinga, Bouwman, Scholte & s'Jacob, 1983; Sumner & Dowler, 1983), such correlations were not recorded in the present study, nor between these criteria and the incidence of root pathogens. Although root discolouration increased with time, percentage of root discolouration did not correlate with yield loss. Alternately, the relationship between visible root discolouration and yield loss may be confounded by other factors such as toxin production by fungi (Mathur, 1968; Cole, Kirksey, Cutler, Doupnik & Peckham, 1973),



therefore, adding to the complexity of the disease. Future research should be aimed at defining the relationship between root rot and yield more accurately, taking into account as wide a range of variables as possible. This could probably be done best under controlled conditions in a greenhouse, before verifying results under field conditions.

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Table 1. Isolation frequencies of fungi isolated from maize roots at four localities

Fungus	Locality								LSD <sup>*</sup> (Lesions)	LSD <sup>*</sup> (Localities)	
	Potchefstroom		Viljoenskroon		Bloekomspruit		Mmabatho			**	***
	DR	CR	DR	CR	DR	CR	DR	CR			
<i>Phoma</i> spp.	241 (5.49)	359 (5.89)	1450 (7.28)	1548 (7.35)	508 (6.23)	663 (6.50)	234 (5.46)	255 (5.55)	n/s	0.38	0.31
<i>Trichoderma</i> spp.	87 (4.48)	105 (4.66)	683 (6.53)	393 (5.98)	319 (5.77)	116 (4.76)	92 (4.53)	67 (4.22)	0.20	0.50	0.41
<i>Alternaria</i> spp.	0 (0)	0 (0)	21 (3.09)	54 (4.01)	0 (0)	0 (0)	60 (4.11)	53 (3.99)	n/s	0.22	0.18
<i>Curvularia</i> spp.	0 (0)	0 (0)	112 (4.43)	106 (4.67)	219 (5.39)	292 (5.68)	15 (2.77)	6 (1.95)	n/s	0.30	0.24
<i>T. terricola</i>	0 (0)	0 (0)	132 (4.89)	144 (4.98)	0 (0)	0 (0)	134 (4.91)	141 (4.96)	n/s	0.27	0.22
<i>M. phaseolina</i>	158 (5.07)	161 (5.09)	167 (5.12)	118 (4.78)	4 (1.39)	7 (2.08)	960 (6.87)	685 (6.53)	0.15	0.38	0.31
<i>E. pedicellatum</i>	661 (6.50)	203 (5.32)	150 (5.02)	55 (4.03)	138 (4.93)	47 (3.89)	0 (0)	0 (0)	0.15	0.38	0.31
<i>F. oxysporum</i>	712 (6.57)	482 (6.18)	1056 (6.96)	827 (6.72)	850 (6.75)	496 (6.21)	497 (6.21)	492 (6.20)	0.14	0.36	0.30
<i>F. moniliforme</i>	73 (4.30)	77 (4.36)	82 (4.42)	63 (4.16)	111 (4.72)	86 (4.47)	124 (4.83)	80 (4.39)	n/s	n/s	n/s
<i>F. chlamydosporum</i>	424 (6.05)	742 (6.61)	174 (5.16)	232 (5.45)	31 (3.47)	49 (3.91)	140 (4.95)	163 (5.10)	n/s	0.44	0.36
<i>F. equiseti</i>	215 (5.38)	307 (5.73)	636 (6.46)	815 (6.70)	250 (5.53)	378 (5.94)	774 (6.65)	855 (6.75)	0.16	0.42	0.40
<i>F. culmorum</i>	18 (2.94)	29 (3.40)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	n/s	0.13	0.11
<i>F. graminearum</i>	0 (0)	0 (0)	89 (4.50)	66 (4.20)	0 (0)	0 (0)	0 (0)	0 (0)	n/s	0.20	0.16
<i>F. compactum</i>	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	92 (4.53)	89 (4.50)	n/s	0.20	0.16
<i>F. sambucinum</i>	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	79 (4.38)	104 (4.65)	n/s	0.21	0.17

\* Differences ( $P < 0.05$ ) based on  $\text{Log}(x + 1)$  transformation indicated in brackets

\*\* LSD (localities) for Potchefstroom

\*\*\* LSD (localities) for Viljoenskroon, Bloekomspruit and Mmabatho

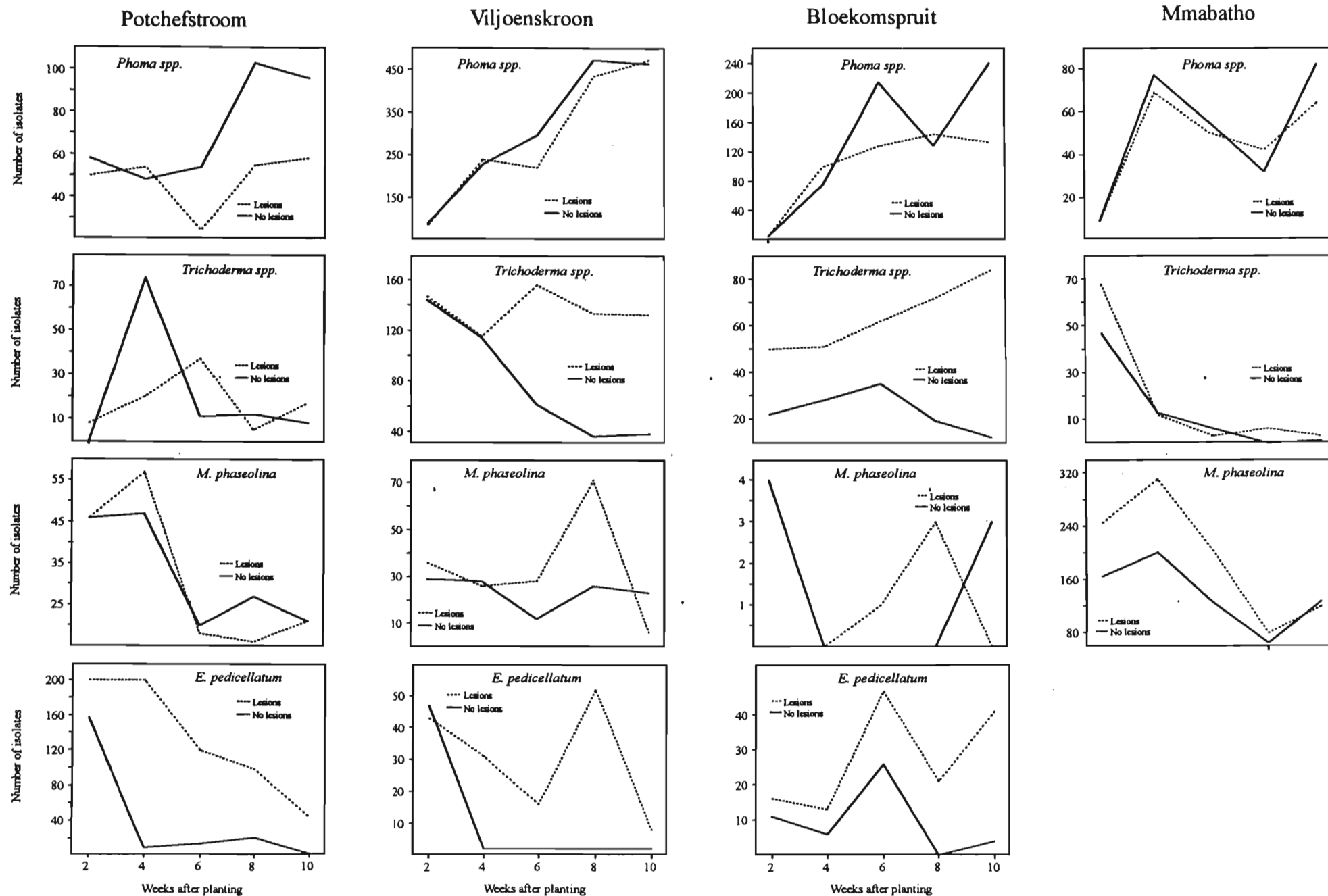


Fig. 1. Frequency of the most common fungi isolated from maize roots at various times after planting at four localities

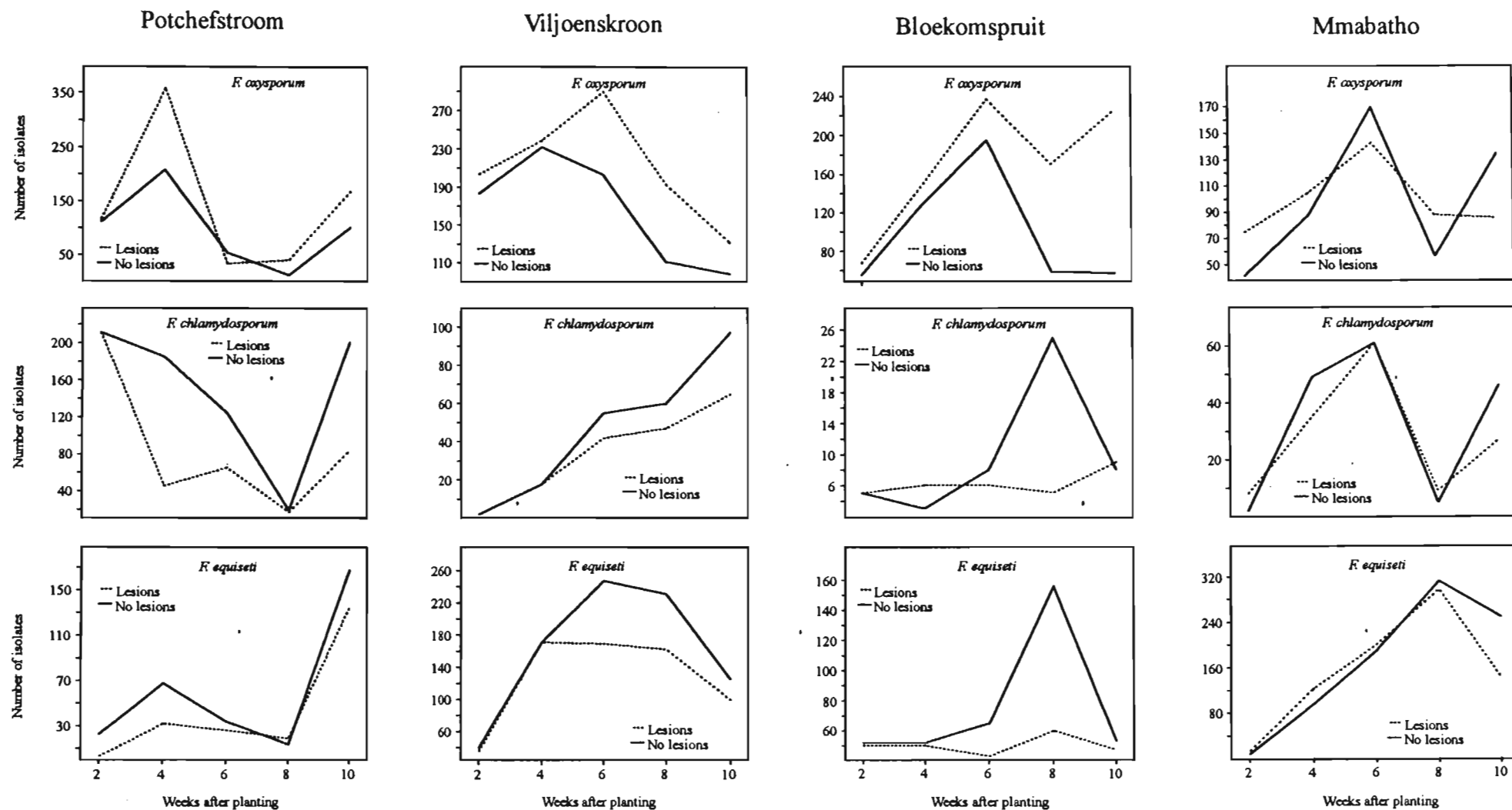


Fig. 1. (Continued) Frequency of the most common fungi isolated from maize roots at various times after planting at four localities.



## CHAPTER 3

# THE EFFECT OF WATER STRESS ON THE INCIDENCE OF MAIZE ROOT ROT AND THE SPECTRUM OF CAUSAL FUNGI

### ABSTRACT

Numerous soilborne fungi have the ability to infect maize roots. Environmental factors can play a major role in the composition of fungi involved and incidence of this disease complex. The isolation frequency of maize root fungi was determined in an existing long-term water stress trial at Potchefstroom during the 1992/93 and 1993/94 seasons. Canvas covers were placed on the ground to induce water stress. Treatments included no stress (irrigation), normal rainfall, stress until flowering, stress after flowering until mid grain-fill, stress after flowering and total stress. Rainfall differed significantly between seasons and results were more obvious during the drier 1992/93 season. Fungi isolated from discoloured and clean root tissues were identified and quantified. The most frequently isolated fungi were *Fusarium equiseti*, *F. oxysporum*, *Exserohilum pedicellatum* and *Macrophomina phaseolina*. Fungi classified as maize root pathogens were isolated at higher frequencies in the no stress and normal rainfall treatments as opposed to the total stress treatment. These fungi occurred early in the season in actively growing juvenile root tissue. Root colonizers were isolated throughout the season whereas others occurred near the end of the season and were associated with senescing root tissue. Regression analysis was conducted to determine the relationship between the water stress index and isolation frequency of the major root fungi involved. A positive, linear relationship between the water stress index and the isolation frequency of *M. phaseolina* was obtained. Negative, non-linear relationships were recorded,

however, between the water stress index and isolation frequencies of *E. pedicellatum*, *F. oxysporum*, *F. moniliforme* and *F. equiseti* suggesting that mild water stress may promote root colonization.

## INTRODUCTION

The nature of the maize (*Zea mays* L.) root rot complex is dependent, to a considerable extent, on prevailing environmental conditions (Whitney & Mortimore, 1957; 1961). Furthermore, Mortimore & Ward (1964) stated that root rot in southwestern Ontario could not be attributed to a single causal organism. Similarly, Chambers (1987a; b) concluded that root rot of maize in South Africa is caused by a complex of soilborne fungi.

Root and stalk rot of maize are regarded as senescence diseases induced by decreasing plant vigour. The latter may be affected by any of a number of environmental stress factors, which in turn, predispose roots to infection (Mortimore & Ward, 1964). Cook & Papendick (1972) reported a close correlation between the influence of water potential on disease development and its concomitant effect on growth of the causal fungi. They found that most soilborne pathogens survive and infect plants primarily in the tillage layer (upper 24 cm of soil) where drying occurs first and most intensively. Pathogens are generally exposed to considerably lower water potentials than the host because roots penetrate the more moist horizons of the soil profile. Cook (1973) found growth of many soilborne plant pathogens to be stimulated rather than inhibited at lower osmotic water potentials. In contrast, Sumner (1968), using root weight as a parameter of root rot, found that root weight was significantly greater in low-moisture treatments, indicating less root rot in dry conditions. Root systems

of plants grown in saturated soils, however, were severely discoloured and decayed.

This study was, therefore, initiated to determine the effect of water stress treatments on the incidence of root rot and the spectrum of fungi isolated.

## MATERIALS AND METHODS

The study was conducted at Potchefstroom during the 1992/93 and 1993/94 plant growth seasons, using an existing long-term water stress field trial. The trial was laid out on a Hutton soil with a clay content of 35%. Fertilizer was applied according to the recommendation of the FSSA-fertilizer (Buys, 1988) manual with a side dressing of 28 kg ha<sup>-1</sup> nitrogen, four weeks after planting. Atrazine was applied to control weeds.

The trial consisted of five water stress treatments replicated four times in a randomized block design. Each treatment consisted of six rows, 28 m in length, spaced 1.5 m apart. Maize cultivars PAN473 (1992/1993) and PAN5206 (1993/1994) were planted at densities of 15000 plants ha<sup>-1</sup>. All treatments commenced with a wet soil profile and included the following: (A) no stress (irrigation), (B) stress until flowering, (C) stress after flowering, (D) stress after flowering until mid grain-fill, (E) total stress and (F) normal rainfall. Rainfall during the two seasons were (509 mm and 819 mm respectively). Water stress treatments were induced using canvas covers placed on the ground to induce soil water stress according to treatment specifications. Irrigation was applied according to measurements obtained from infra red thermometers. During the second season no irrigation was applied since sufficient rainfall was received.

Plants were sampled every fortnight commencing four weeks after planting until 50% tasselling. Three plants per plot per season (total = 420) were dug out at each sampling date, endeavouring to maintain as much as possible of the root system. Recovered roots were thoroughly washed in water to facilitate the removal of all soil and debris. The percentage root rot was visually assessed. Roots with visible discolouration (DR) were cut into 3 mm segments and surface-sterilised in 3.5 % NaOCl for 1 min. After sterilisation, root segments were rinsed twice in sterile distilled water. Fifty root segments per sample were plated out onto each of Potato Dextrose Agar, Malt Extract Agar and Potato Carrot Agar for isolation of root fungi. Root isolates were cultured for two days at 25°C and were then reisolated, identified and quantified. *Fusarium* isolates were plated out onto Carnation Leaf Agar for identification. From each sample the same number of root segments with no visible discolouration (CR) was similarly treated and included as controls. Differences in isolation frequency between DR and CR root samples were used to distinguish between fungi that may be regarded as pathogens and to root colonizers.

Fungal isolation frequencies were compared using analysis of variance. Scheffe's LSD was used to determine differences between water stress treatments and isolation frequency in DR and CR root tissues. Regression analysis was used to determine the relationship between isolation frequency of fungi from infected maize roots and a water stress index (WSI) determined using measurements obtained from a neutron water meter.

## RESULTS

The spectrum of fungi isolated from maize roots is presented in Table 1. *Fusarium equiseti*

(Corda) Sacc., *F. oxysporum* Schlecht emend. Snyder & Hans., *Exserohilium pedicellatum* (Henry) Leonard & Suggs and *Macrophomina phaseolina* (Tassi) Goid were the most frequently isolated. The incidence of *Phoma* spp., *E. pedicellatum*, *F. oxysporum*, *F. moniliforme* and *F. equiseti*, pooled over all sample dates, was significantly ( $P < 0.05$ ) higher in the DR than in the CR root samples during both seasons, while *Trichoderma* spp. only differed significantly ( $P < 0.05$ ) during the first season (Fig. 1). No significant difference in isolation frequency between DR and CR root samples was found with *M. phaseolina* and *F. chlamydosporum* Wallenw. & Reiking (Fig. 1).

During the 1992/93, significant ( $P < 0.05$ ) treatment differences were recorded with *Trichoderma* spp., *Phoma* spp., *E. pedicellatum* and *F. moniliforme*. Maize plants in the no stress (A) and normal rainfall (F) treatments were more susceptible to infection than plants that were totally stressed (E), particularly with regard to isolation frequencies of *E. pedicellatum*, *F. oxysporum* and *F. moniliforme* (Fig. 1). Similarly, a low incidence of isolates occurred in the pre-flowering moisture stress treatments (B), whereas a higher incidence of isolates was found in the post-flower water stress treatments (C and D) (Fig. 1). These tendencies were, however, not as prominent during the 1993/94 season and significant treatment effects were only recorded for *F. chlamydosporum*.

Time of isolation had a significant ( $P < 0.05$ ) effect on isolation frequency of major fungi (Fig. 2). *Trichoderma* spp., *E. pedicellatum*, *F. oxysporum*, *F. moniliforme* and *F. chlamydosporum* were isolated early in the season reaching a maximum at six weeks after planting with a subsequent rapid decline. *M. phaseolina*, however, was isolated at a high frequency early in the first, drier season and late in the second, wetter season, while the

inverse occurred for *F. equiseti* (Fig. 2).

A positive linear relationship between the WSI and isolation frequency of *M. phaseolina* (Fig. 3a) was obtained, indicating a tendency for increased infection under increasing water stress. Non-linear relationships between the WSI and isolation frequencies of *E. pedicellatum* (Fig. 3b), *F. oxysporum* (Fig. 3c), and *F. moniliforme* (Fig. 3d) were recorded which indicated an increase in root colonization during slight water stress. Isolation frequency decreased rapidly as water stress increased. *F. equiseti* (Fig. 3e) gave a similar non-linear relationship, but reached a maximum isolation frequency when water stress was intermediate.

First and secondary polynomial regression analyses were used to determine the relationship between isolation frequency of primary isolates during 1992/93 and a water stress index (WSI). Quantification of the water stress index was based on the CERES Maize-V2.10 model (Ritchie, Singh, Godwin & Hunt, 1992), using an equation, for determining water stress, from Stewart & Hagan (1973). This equation correlated significantly with relative percentage rainfall and yield (Fig. 4).

A sufficient WSI was not obtained during the second season and may be ascribed to the higher rainfall received. Therefore, regression analysis was not carried out for the season. No significant correlation coefficients were obtained between isolation frequencies of the major root rot fungi and percentage root discolouration.



## DISCUSSION

Research on the etiology of maize root rot has indicated that no single causal organism is involved, but rather a complex of organisms (Chambers 1987a, b; Mortimore & Ward, 1964; Hornby & Ullstrup, 1967). The spectra of fungi isolated in this study corresponded with that isolated from maize roots elsewhere (Shepherd, Hall & Pendery, 1962; Hornby & Ullstrup, 1967; Du Toit, 1968; 1969; Futrell & Kilgore, 1969; Chambers, 1987a).

It is generally assumed that colonization of maize roots by root pathogens occurs mainly late in the season, emphasizing lodging associated with this disease (Mortimore & Ward, 1964; Thompson, 1972). However, this was not the case with the fungi classified as root rot pathogens in the present study. *E. pedicellatum* occurred early in the season in juvenile tissue and declined as the tissue matured. Greater colonization of actively growing, juvenile tissue than of older, senescent tissue, questions the theory that root rot is a disease of senescing tissue. The late-season decline in *E. pedicellatum* suggested that the organism is unable to compete saprophytically towards the end of the season, emphasizing its pathogenic ability. Similar tendencies were recorded with *F. oxysporum* and *F. moniliforme*. Chambers (1987a) noted that the incidence of *E. pedicellatum* increased shortly after planting and then decreased, whereas *F. moniliforme* decreased as the season progressed. Isolation frequencies of *F. oxysporum* and *F. moniliforme* reached a maximum after *E. pedicellatum* had peaked, raising the question as to whether the latter fungus may act as a predecessor for *Fusarium* spp.. In agreement, Chambers (1987a) found that these differences in isolation frequencies during the season may be due to moisture levels in the soil.

The absence of *E. pedicellatum* during the second season is inexplicable. The difference in maize cultivar and the excessive soil water, which resulted from the higher rainfall may, however, have had a detrimental effect on the incidence of *E. pedicellatum* during 1993/94.

Fungi classified as root-colonizers, e.g. *Phoma* spp., were isolated at high frequencies throughout the growing season. Other fungi such as *F. chlamydosporum*, *F. equiseti* and *F. compactum* occurred late in the season when plants were nearing maturity, suggesting an inability of these fungi to colonize actively growing tissues.

It is generally accepted that water stress plays a major role in the incidence of maize root rot (Du Toit, 1968; Sumner, 1968; Sumner & Bell, 1982), but it seems as if the importance of stress has been overemphasized. No stress (A) and normal rainfall (F) treatments predisposed maize plants more to root rot than the total stress treatment. Sumner (1968) also found that maize root weights were significantly greater in low-moisture treatments, indicating less root rot, whereas the root systems of plants grown in saturated soil were usually severely discoloured and decayed. It has been observed that root diseases occurred more frequently in irrigated field maize than in dry land maize (Sumner & Bell, 1982). No differences were obtained between late-season water stress (C and D) and no stress (A) which suggests that sufficient levels of soil water during the actively growing stages of the plant growth cycle is essential for root colonization. The late season stress seems to be relatively unimportant in the infection process.

*M. phaseolina* was the only fungal species with increased isolation frequency as water stress increased. Young & Kucharek (1977) isolated *M. phaseolina* from maize roots but found



it to occur more frequently in association with root and stalk rot of sorghum under drought stress conditions (Edmunds, 1963; Mughogho & Pande, 1984; Pande, Moghogho & Kurunakar, 1990).

Above-ground symptoms involved in maize root rot are not specific and include wilting, early senescence, stunting and lodging of plants. Root discolouration and root development are the primary criteria for quantification of maize root rot, although the accuracy of these methods is questionable. In this study root discolouration did not correlate with the incidence of root pathogens. Quantification of this disease, therefore, requires further research.

Although maize root rot is caused by numerous fungi, it is evident from this study that, each fungal species reacted differently to water stress and even to normal rainfall conditions. This necessitates further research on fungal species that may be regarded as root pathogens.

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Table 1. Incidence of fungi isolated from maize roots during two consecutive seasons (1992/93 and 1993/94) at Potchefstroom

Fungi	Isolation frequency *
<i>Fusarium equiseti</i>	386.8
<i>Fusarium oxysporum</i>	259.5
<i>Exserohilum pedicellatum</i>	157
<i>Phoma</i> spp.	132
<i>Macrophomina phaseolina</i>	62.8
<i>Trichoderma</i> spp.	41
<i>Fusarium moniliforme</i>	28.5
<i>Fusarium chlamydosporum</i>	26.3
<i>Fusarium culmorum</i>	11.8
<i>Fusarium compactum</i>	5.8
<i>Curvularia</i> spp.	5.3
<i>Bipolaris australiensis</i>	4.5
<i>Thielavia terricola</i>	2.5
<i>Periconia macrospinososa</i>	2
<i>Chaetomium</i> spp.	1.8
<i>Penicillium</i> spp.	1.3
<i>Aspergillus</i> spp.	1.3
<i>Epicoccum purpurascens</i>	1
<i>Neocosmospora africana</i>	0.8
<i>Exserohilum rostratum</i>	0.8

\* Mean of both seasons.

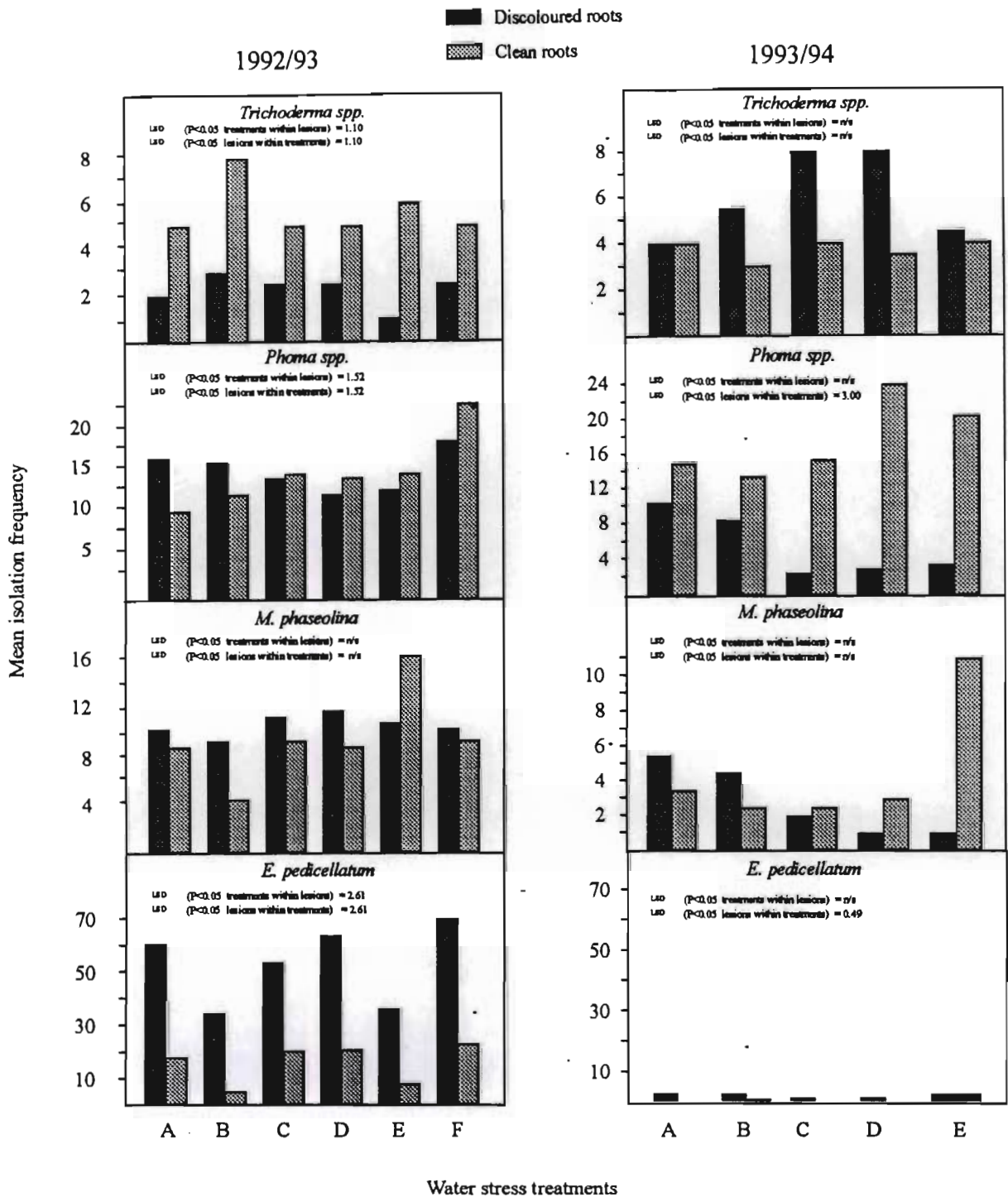


Fig. 1. Isolation frequency of fungi isolated in discoloured root and clean root tissues of maize plants grown under different water stress conditions during the 1992/93 and 1993/94 seasons.

(A= no stress, B= stress untill flowering, C= stress after flowering, D= stress after flowering untill mid grainfill, E= total stress, F= normal rainfall.)



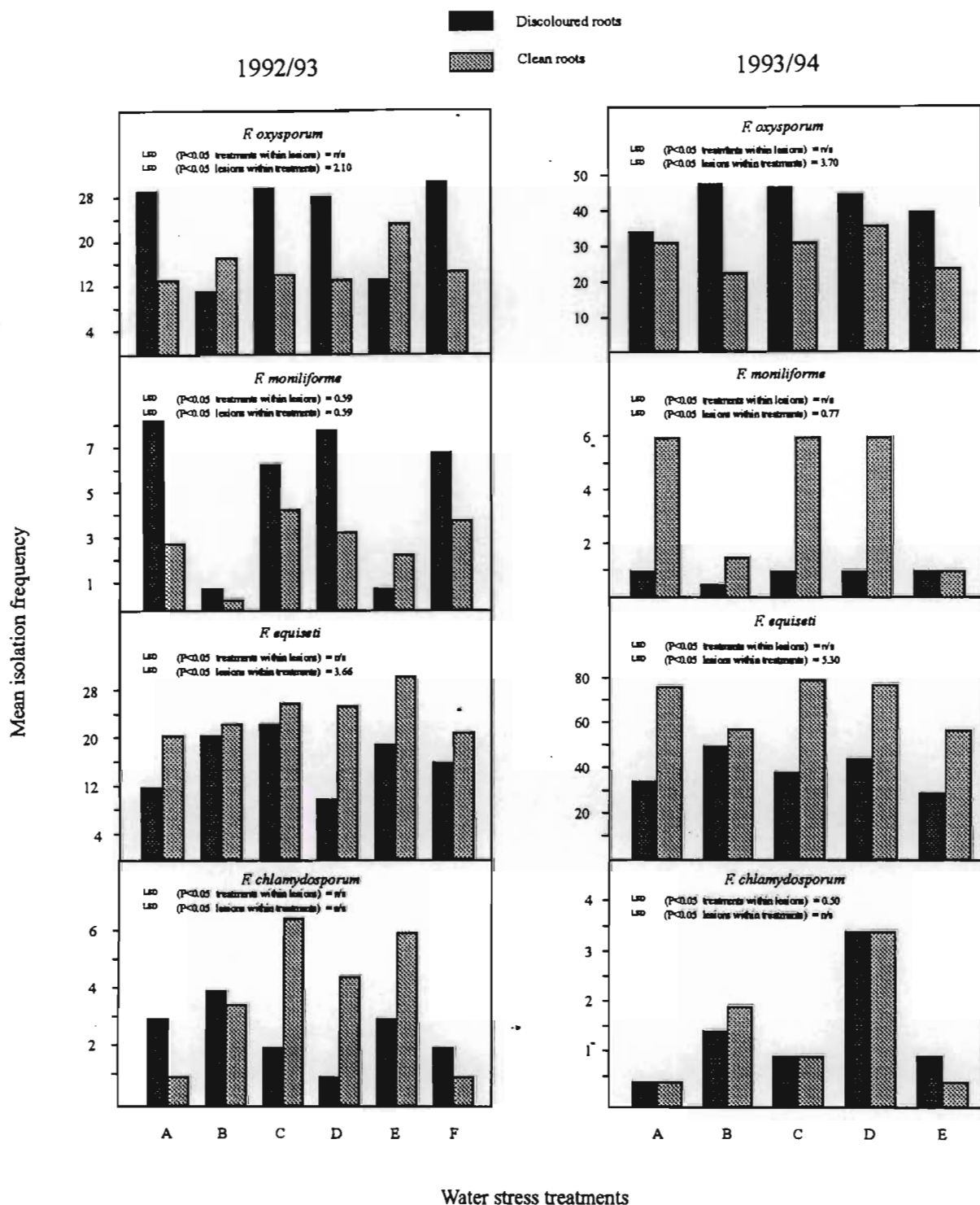


Fig. 1 (Continued). Isolation frequency of fungi isolated in discoloured root and clean root tissues of maize plants grown under different water stress conditions during the 1992/93 and 1993/94 seasons.

(A= no stress, B= stress untill flowering, C= stress after flowering, D= stress after flowering untill mid grainfill, E= total stress, F= normal rainfall.)

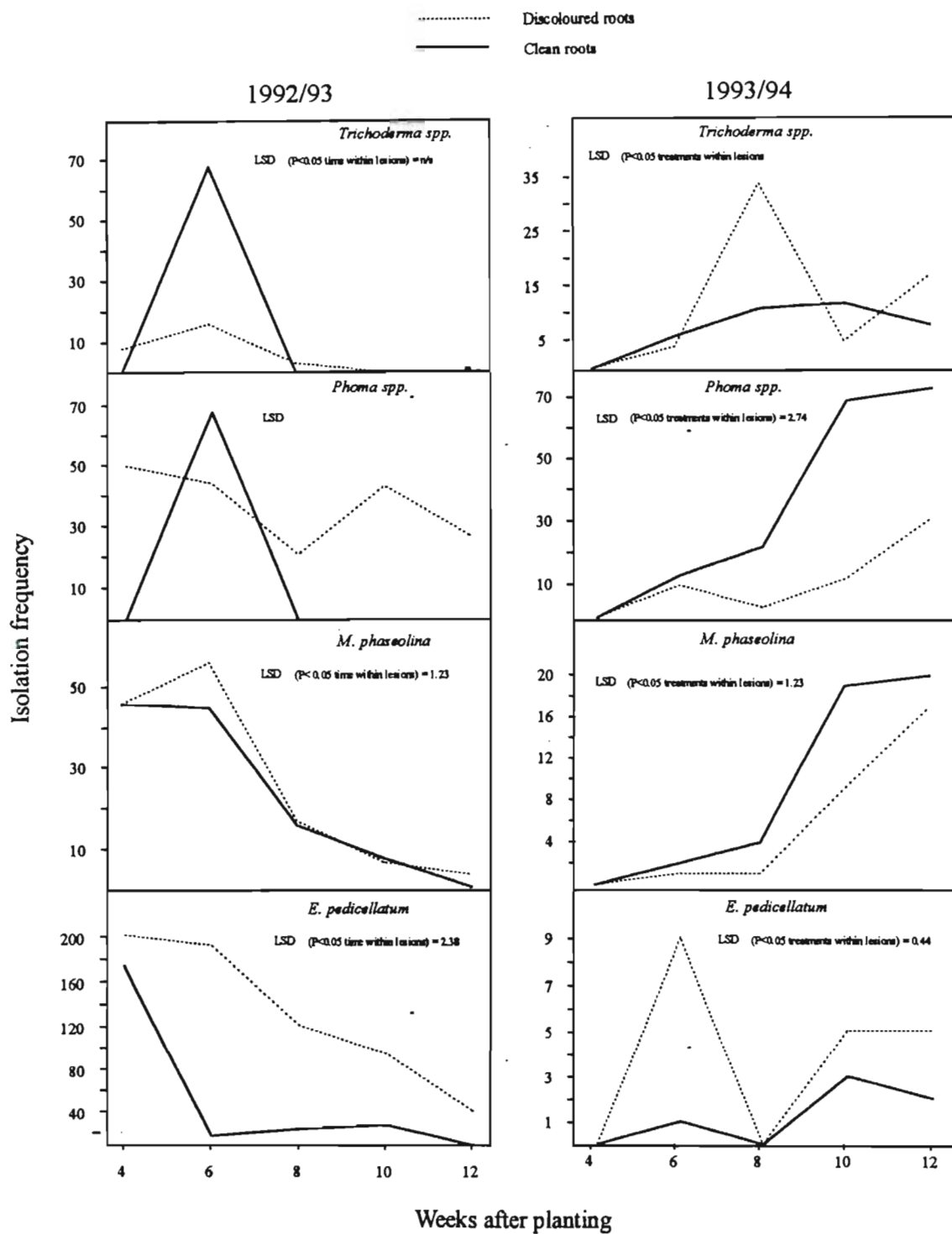


Fig. 2. Isolation frequency of fungi isolated from discoloured and clean root tissue at different maize plant growth stages during the 1992/93 and 1993/94 season.



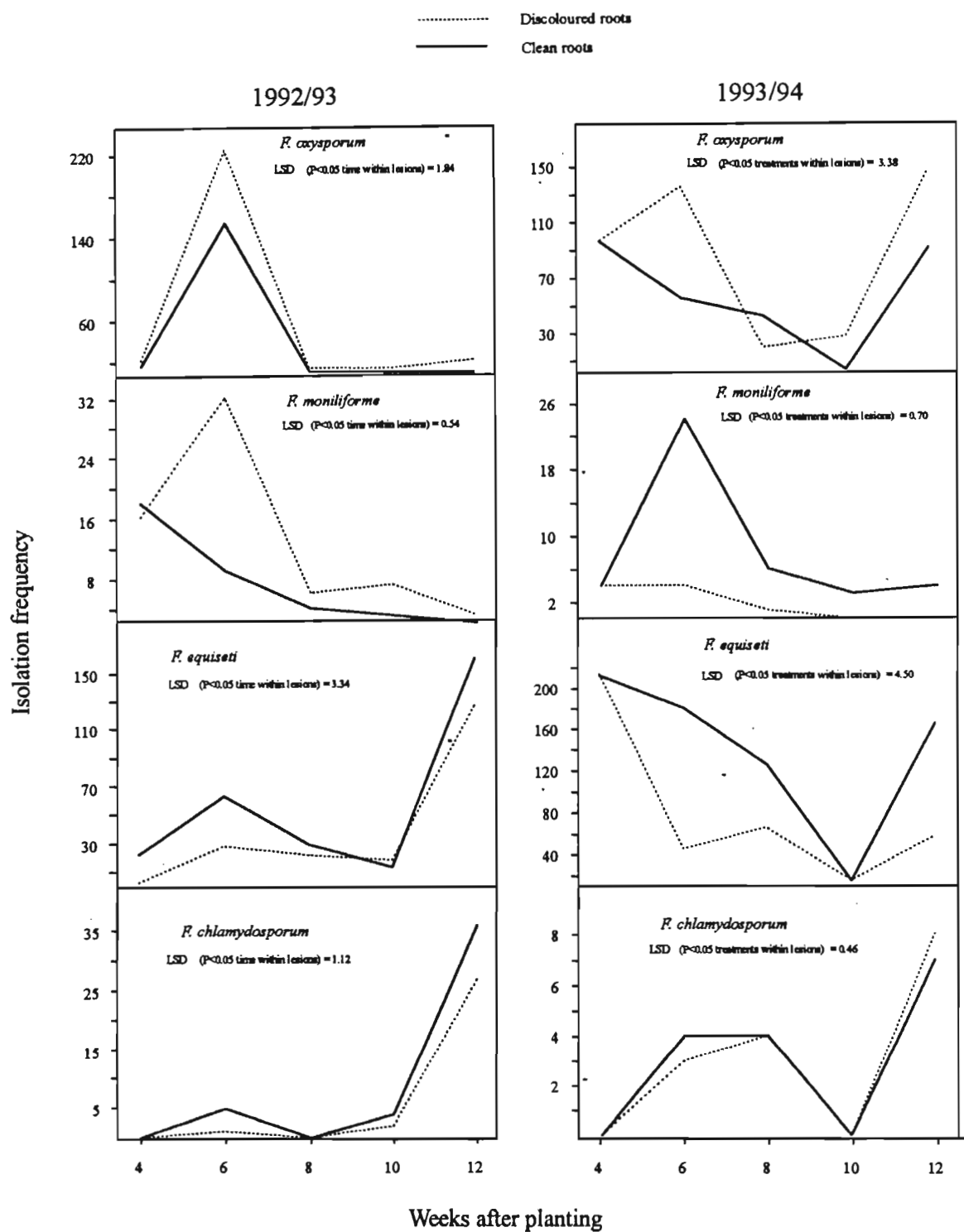


Fig. 2 (Continued). Isolation frequency of fungi isolated from discoloured and clean root tissue at different maize plant growth stages during the 1992/93 and 1993/94 season.

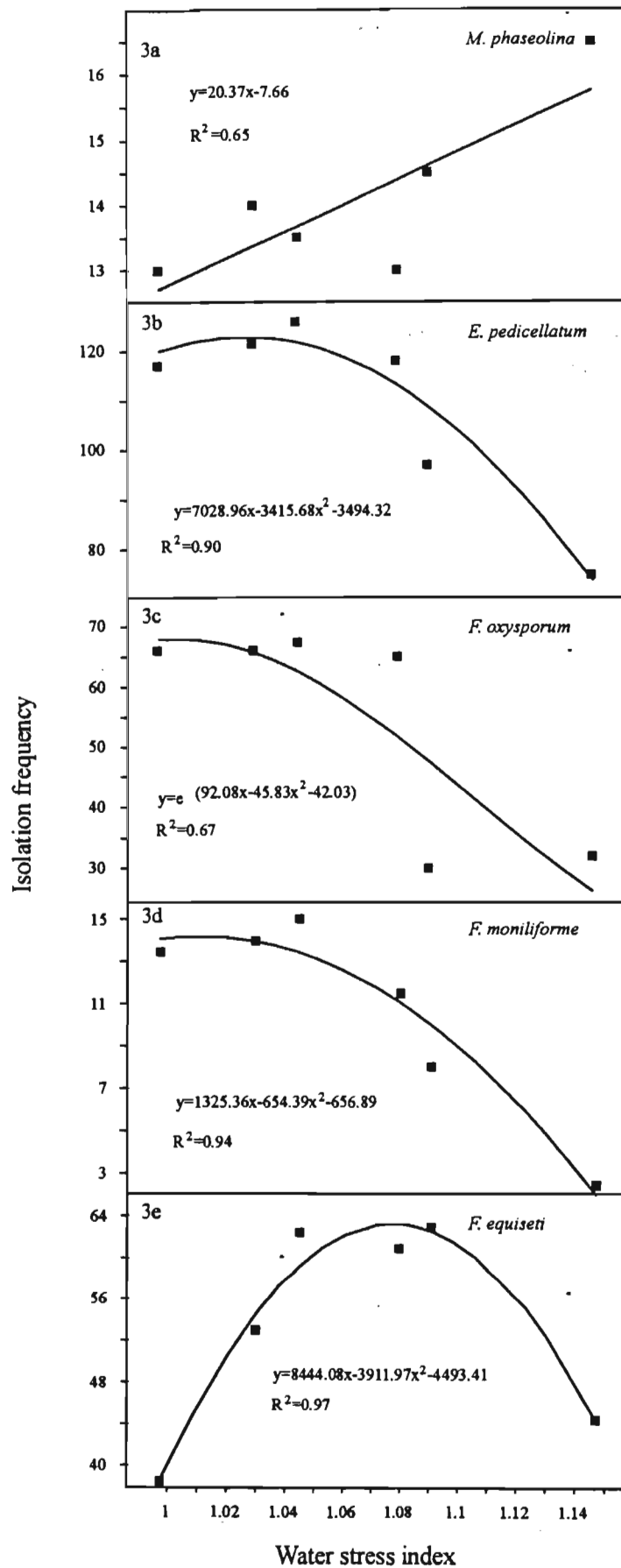


Fig. 3. Relationship between the water stress index and isolation frequency of maize root pathogens.

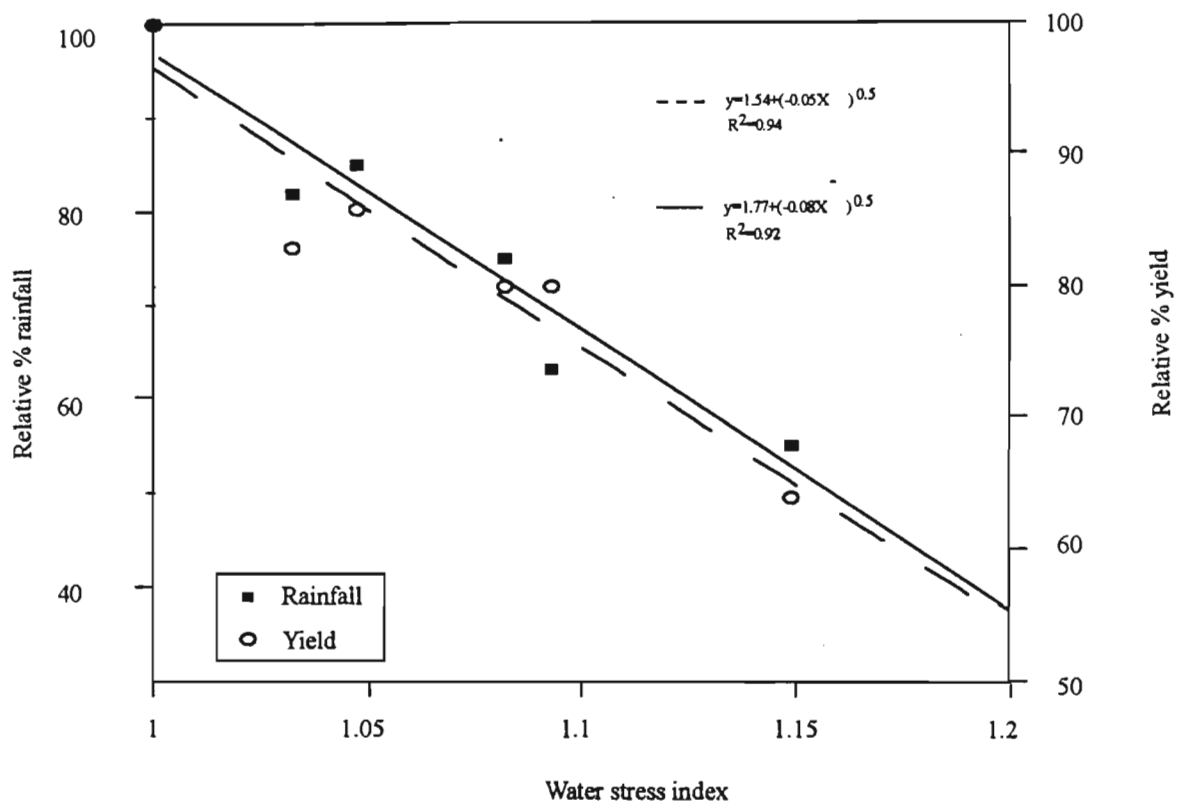


Fig. 4. Relationship between relative rainfall, maize yield and the water stress index.

## CHAPTER 4

### EFFECT OF TILLAGE PRACTICES ON THE INCIDENCE OF MAIZE ROOT ROT AND THE SPECTRUM OF CAUSAL FUNGI

#### ABSTRACT

Maize root rot is widespread in South Africa and various factors may influence disease severity. *Phoma* spp., *Trichoderma* spp., *Fusarium* spp., *Macrophomina phaseolina* and *Exserohilum pedicellatum* were the fungi most commonly isolated from infected roots. The soil microflora is affected by factors affecting the soil profile, and tillage practices may, therefore, affect the incidence of soilborne fungi. Field trials, to determine the effect of tillage practices on disease severity, were carried out at two localities over two seasons. Tillage practices applied were rip, plough, no-till and chisel treatments. Fungi were isolated from discoloured and clean root tissues, identified and quantified. Significant differences in isolation frequencies associated with tillage treatments were recorded for *F. oxysporum* at Bloekomspruit and *Trichoderma* spp., *Alternaria* spp. and *M. phaseolina* at Mmabatho. Different results were obtained regarding the various tillage practices applied at the two localities. The variation in results may be ascribed to the differences in environmental conditions of each locality. Stubble and the extent of soil disturbance, however, seem to play a role in the incidence of the maize root rot complex.

## INTRODUCTION

Root rot occurs widespread in the major maize-producing (*Zea mays* L.) areas of South Africa (Du Toit, 1968). Maize roots are infected by facultatively parasitic fungi which occur in the soil and under the seed coat (Chambers, 1987a). Young & Kucharek (1977) associated five fungal communities in roots and stalks of maize with particular growth stages of the plants. The ability of a fungal species to cause root rot of maize is an isolate rather than a species attribute (Chambers, 1987a). *Fusarium* species seem to be the most common fungi occurring on maize roots. Chambers (1987b) isolated *F. moniliforme* Sheldon and *Exserohilum pedicellatum* (Henry) Leonard & Suggs at higher frequencies from roots than other fungi. Other *Fusarium* species associated with the root rot complex on maize include *F. oxysporum* Schlecht. emend. Snyder & Hans., *F. solani* (Mart.) Appel. & Wollenw. emend. Snyder & Hans., *F. tricinctum* (Corda) Sacc., and *F. proliferatum* (Matsushima) Nirenberg (Warren & Kommedahl, 1973; Zummo, 1984; Leslie, Pearson, Nelson & Toussoun, 1990). *Rhizoctonia* spp. (Sumner & Bell, 1982), *Sclerotium* spp. (Sumner, Bell & Huber, 1979), *Pythium graminicola* Subr. (Rao, Schmitthenner, Caldwell & Ellet, 1978) and *Phialophora zeicola* Deacon & Scott (Deacon & Scott, 1983) are also included in the root rot complex.

The soil microflora is affected by factors affecting the soil, and different tillage practices will, therefore, affect the biocoenoses of the soil (Herman, 1984). *Fusarium* spp. occurred more frequently in unploughed soil which may have a bearing on the extent to which *Fusarium* invades healthy roots (Herman, 1984). *Rhizoctonia solani*, on the other hand, proved to be more common in cereals grown under conservation tillage (Weller, Cook,

MacNish, Bassett, Powelson & Petersen, 1986). Sumner, Gascho, Johnson, Hook & Threadgill (1990) found that root rot severity was reduced in soil prepared with a mouldboard plough compared with discing, chiselling, or in-row subsoiling, although differences were not significant. Tillage practices in South Africa range from conventional tillage to no-tillage. The objective of this study was to investigate the incidence of root rot in maize and the spectrum of root rot pathogens under different tillage practices.

## MATERIALS AND METHODS

Field trials were conducted at Bloekomspruit and Mmabatho during the 1992/93 and 1993/94 seasons. Both localities have Hutton soil types with clay contents of 23% and 10%, respectively. Localities were selected on the basis of an ongoing long-term tillage trial at Bloekomspruit, whereas severe lodging occurred at the Mmabatho trial site.

A randomised block design with three replicates was used with tillage as the plot factor at both localities. Plots at Bloekomspruit consisted of 9 rows, 135 m in length and spaced 1.5 m apart. At Mmabatho, plots consisted of 6 rows, 200 m in length and spaced 2.2 m apart. An inter-row spacing of 30 cm was used at both localities.

Tillage practices applied at Mmabatho were deep and shallow chisel plough (DCP and SCP), deep and shallow mouldboard (DMP and SMP) and rip. Since most lodging occurred with SMP during the first season it was replaced with a rip-on-row treatment to which lime was applied in a band ( $2\text{ t ha}^{-1}$ ) during the 1993/94 seasons. In addition to DMP, SMP and rip treatments, disc and no-till (NT) plots were also included at Bloekomspruit. Trials were

fertilised according to the recommendations of the FFSA-fertilizer manual at each locality. The maize cultivar PAN473 was planted at Mmabatho and PAN6528 at Bloekomspruit. Plants were maintained by applying insect and weed control as required until sampling.

Sampling commenced four weeks after planting at fortnightly intervals until 50% tasselling. Five plants per plot were dug out endeavouring to maintain as much as possible of the root system. A total of 750 root systems was sampled at each locality during the two seasons. Roots of each sample were thoroughly washed in water to remove soil and debris. Visual assessments of the percentage root rot, based on root discolouration, were carried out during both seasons at Bloekomspruit, but only during the second season at Mmabatho.

Discoloured root tissues (DR) were cut into 3 mm segments and surface-sterilised in 3,5% NaOCl for 1 min. After being rinsed twice in sterile distilled water, 50 root segments per sample were plated out onto each of Potato Dextrose Agar, Malt Extract Agar and Potato Carrot Agar for identification. Isolated *Fusarium* spp. were cultured on Carnation Leaf Agar for identification. Cultures were incubated at 25°C for three days, after which fungi isolated from maize roots were identified and quantified. Roots with no visible discolouration (CR) from the same samples and in equal numbers, were similarly treated and included as controls.

At both localities the dry mass of the aerial plant parts was determined after each sample date during the first season by drying the plant material at 60°C until mass was constant. Yield was determined at plant maturity on the remaining plants per plot and expressed as kg grain per treatment.

Analysis of variance was used to determine differences between tillage practices and isolation frequency of fungi. The correlation between the incidence of fungi isolated from maize roots and both root discolouration and dry-mass was determined.

## RESULTS

The spectra of fungi isolated from maize roots at Bloekomspruit and Mmabatho corresponded with regard to composition, but differed in isolation frequencies (Tables 1 and 2, respectively). *F. oxysporum* and *Phoma* spp. were isolated at the highest frequencies at Bloekomspruit, while *F. equiseti* (Corda) Sacc. and *Macrophomina phaseolina* (Tassi) Goid. were most common in Mmabatho. Significant differences ( $P < 0.05$ ) in isolation frequencies between discoloured (DR) and clean root (CR) tissues were found for *Phoma* spp., *Trichoderma* spp., *E. pedicellatum*, *F. oxysporum* and *F. equiseti* at Bloekomspruit and with *Thielavia terricola* (Gilman & Abbot) Emmons and *M. phaseolina* at Mmabatho.

Isolation frequencies of *F. oxysporum* and *F. equiseti* differed significantly ( $P < 0.05$ ) between tillage treatments at Bloekomspruit (Table 1). Significant differences ( $P < 0.05$ ) between isolation frequency and tillage treatments were recorded for *Trichoderma* spp., *Alternaria* spp. and *M. phaseolina* at Mmabatho (Table 2). The remaining fungi seemed to be unaffected by tillage practices.

No significant ( $P < 0.05$ ) correlations were found between the isolation frequency of any pathogen and percentage root discolouration at Bloekomspruit during both seasons, whereas *F. oxysporum* and *F. equiseti* showed a significant ( $P < 0.05$ ) negative correlation with root



discolouration during the second season at Mmabatho (Table 3). The isolation frequencies of *Curvularia* spp. and *T. terricola* correlated significantly ( $P < 0.05$ ) with dry mass of aerial plant parts at Bloekomspruit at Mmabatho, respectively (Table 4). The remaining fungi did not correlate significantly with dry mass.

Root discolouration differed significantly ( $P < 0.05$ ) between tillage treatments at Bloekomspruit, but not at Mmabatho (Fig. 1). The shallow chisel plough treatment was associated with the least root discolouration, in comparison with rip, DMP and NT treatments. No significant differences between root discolouration and tillage treatments were recorded at Mmabatho. However, DCP tended to cause more discolouration in comparison with the remaining tillage treatments (Fig. 1).

Significant ( $P < 0.05$ ) differences were recorded between yield and tillage treatments at Bloekomspruit for both seasons (Fig. 2). The NT treatment had the lowest yield during both seasons, while the rip and DMP treatments showed the highest yield during the first season. No significant differences in yield occurred between tillage treatments at Mmabatho.

## DISCUSSION

Differences between isolation frequencies of fungi may be ascribed to environmental differences experienced at the two localities. At Bloekomspruit, rainfall of 486 mm and 435 mm was recorded for the 1992/93 and 1993/94 seasons, respectively. Rainfall at Mmabatho was 184 mm and 343 mm for the two seasons, respectively.

Inconsistent results were obtained at Mmabatho regarding the effect of tillage treatments on the isolation frequency of fungi. The isolation frequency of *M. phaseolina* decreased in soil which was most disturbed by tillage treatments, whereas chisel tillage treatments tended to decrease the incidence of *Alternaria* spp.. *M. phaseolina* is a known pathogen of maize roots, occurring more frequently under drought stress conditions (Pande, Mughogho & Kurunakar, 1991). Prevalence of this fungus at Mmabatho may therefore, be enhanced by the low rainfall associated with this area. *E. pedicellatum*, on the other hand, has been found to decrease when water stress prevails (Chapter 3), hence its absence at Mmabatho as opposed to Bloekomspruit.

Soil compaction occurs commonly throughout South Africa (Van Huysteen, 1994: pers. comm.)<sup>1</sup> and appropriate soil profile modifications and soil management practices have to be applied to optimise soil physical conditions. Compacted soils induce poor root penetration and result in distorted roots. Under stressed soil conditions roots are

predisposed to colonization by soilborne pathogens (Van Huysteen, 1994: pers. comm.). Sumner & Bell (1986) found that tillage practices may influence residue distribution and, hence, soil compaction. Furthermore, the effect of maize crown and brace root rot on yield reduction may be greater if root growth is restricted by soil compaction.

At Bloekomspruit the soil was disturbed most by the SCP and disc treatments and least by the rip treatment. *F. oxysporum* was isolated at a higher frequency in the SCP and disc

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<sup>1</sup> Dr. L. van Huysteen, Nietvoorbij Institute for Viticulture and Oenology, Stellenbosch.

treatments, but with no differences between the rip, DMP and NT treatments. Herman (1984) found a higher occurrence of *Fusarium* spp. in the rhizosphere, rhizoplane and roots of wheat in unploughed soil than in ploughed (tilled) soil. However, Lipps & Deep (1991) isolated *Fusarium* spp. more frequently from rotted stalks taken from conventionally tilled than from no-till fields. Although no-till practices conserve soil water, root penetration may be restricted and stressed, accounting for the high occurrence of *F. oxysporum*. Reduced-tillage practices are known to cause a decline in the total number of fungi with increasing depth, whereas with ploughed systems, fungi are more evenly distributed in the soil (Norstadt & McCalla, 1968; Sumner, Doupnik (Jr.) & Boosalis, 1981). Therefore, due to plant residues in the upper soil layers, more fungi and micro-organisms will be present in soil subjected to reduced-tillage practices compared to ploughed systems (Doran, 1980).

Patrick, Toussoun & Snyder (1963) found that minimum tillage concentrates plant residues in the top 10 to 15 cm of the soil. Similarly, Sumner, *et al* (1981) recorded a decrease in soilborne disease incidence when soil residue is ploughed in. Contradictory results between the two localities were found regarding tillage practices and incidence of maize root fungi in this study. Stubble concentrations on the ground, acquired with different tillage treatments at Bloekomspruit, had no effect on the incidence of the fungi. No differences between the spectra of fungi isolated was obtained, irrespectively of whether the stubble remained above-ground or within the soil, except with the SCP treatment. The soil profile at Bloekomspruit was relatively wet during the two seasons and that may be the reason why no differences were recorded. However, at Mmabatho the seasons were relatively dry and the spectrum of fungi involved tended to decrease when stubble was buried with the plough treatments. Stubble on the soil surface conserves water and creates a more favourable

environment for soilborne fungi. Interactions between tillage practices and incidence of maize root fungi seem promising and need to be investigated with regard to developing a disease control system.

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Table 1. Mean number of fungi isolated from discoloured (DR) and clean root (CR) maize samples from different tillage treatments at Bloekomspruit during the 1992/93 and 1993/94 seasons

Fungi		Tillage treatments <sup>1</sup>					Significant level <sup>2</sup>
		Rip	DMP	SCP	NT	Disc	
<i>Trichoderma</i> spp.	DR	65	67	52	81	70	0.717
	CR	21	23	22	18	35	
<i>Phoma</i> spp.	DR	141	138	135	125	115	0.885
	CR	164	130	105	110	192	
<i>Curvularia</i> spp.	DR	39	53	60	59	47	0.521
	CR	69	67	65	76	81	
<i>Exserohilum pedicellatum</i>	DR	30	33	40	14	35	0.345
	CR	14	6	14	5	8	
<i>Fusarium oxysporum</i>	DR	196	184	133	222	174	0.012
	CR	120	122	31	110	94	
<i>Fusarium moniliforme</i>	DR	20	29	34	24	18	0.619
	CR	11	13	6	19	36	
<i>Fusarium chlamydosporum</i>	DR	5	3	10	14	4	0.580
	CR	9	17	10	11	8	
<i>Fusarium equiseti</i>	DR	30	68	72	72	33	0.002
	CR	72	107	64	86	46	

<sup>1</sup> DMP = Deep mouldboard plough, SCP = Shallow chisel plough, NT = No-Tillage

<sup>2</sup> Comparison of tillage treatments



Table 2. Mean number of fungi isolated from discoloured root (DR) and clean root (CR) maize samples from different tillage treatments at Mmabatho during the 1992/93 and 1993/93 seasons

Fungi		Tillage treatments <sup>1</sup>						Significant level <sup>2</sup>
		DMP	DCP	SCP	Rip	SMP93	ROR94	
<i>Trichoderma</i> spp.	DR	6	32	6	9	39	0	0.050
	CR	5	34	6	4	18	0	
<i>Phoma</i> spp.	DR	77	58	38	61	13	40	0.474
	CR	76	59	58	71	35	26	
<i>Curvularia</i> spp.	DR	4	1	1	7	0	2	0.204
	CR	2	0	2	1	0	3	
<i>Alternaria</i> spp.	DR	17	21	5	6	0	11	0.050
	CR	12	21	6	8	0	16	
<i>Macrophomina phaseolina</i>	DR	142	164	230	255	154	112	0.001
	CR	67	139	175	172	72	117	
<i>Thielavia terricola</i>	DR	34	9	24	26	7	43	0.163
	CR	21	25	27	47	13	26	
<i>Fusarium oxysporum</i>	DR	133	150	87	162	73	53	0.401
	CR	136	128	134	154	111	41	
<i>Fusarium moniliforme</i>	DR	10	35	7	52	7	20	0.287
	CR	22	16	23	13	9	9	
<i>Fusarium chlamydosporum</i>	DR	31	29	38	33	34	2	0.812
	CR	16	23	42	48	54	0	
<i>Fusarium equiseti</i>	DR	250	365	294	303	254	34	0.904
	CR	297	297	294	307	330	38	

<sup>1</sup> DMP=Deep mouldboard plough, DCP=deep chisel plough, SCP=shallow chisel plough, SMP(93)=Shallow mouldboard plough in 92/93 season, ROR(94)=Rip-on-row with lime applied in a band in 93/94 season.

<sup>2</sup> Comparison of tillage treatments.

Table 3. Correlation coefficients showing the relationship between isolation frequency of fungi from maize roots and root discolouration during the 1992/93 and 1993/94 seasons at Bloekomspruit and during the 1993/94 season at Mmabatho

Fungi	Bloekomspruit (92/93)	Bloekomspruit (93/94)	Mmabatho (93/94)
<i>Phoma</i> spp.	0.045	0.014	-0.083
<i>Trichoderma</i> spp.	0.218	0.27	-0.002
<i>Curvularia</i> spp.	-0.227	-0.196	0
<i>Alternaria</i> spp.	0	0	-0.158
<i>Macrophomina</i> <i>phaseolina</i>	0	0.275	-0.009
<i>Exserohilum</i> <i>pedicellatum</i>	-0.291	-0.185	0
<i>Thielavia</i> <i>terricola</i>	0	0	-0.042
<i>Fusarium</i> <i>oxysporum</i>	0.492	0.47	-0.536*
<i>Fusarium</i> <i>moniliforme</i>	-0.011	0	0
<i>Fusarium</i> <i>chlamydosporum</i>	0.036	0.076	0.144
<i>Fusarium</i> <i>equiseti</i>	-0.166	0.184	-0.534*

\*  $P < 0.05$

Table 4. Correlation coefficients showing the relationship between isolation frequency of fungi isolated from maize roots and aerial dry mass of maize at Bloekomspruit and Mmabatho during the 1992/93 season

Fungi	Bloekomspruit	Mmabatho
	(92/93)	(92/93)
<i>Phoma</i> spp.	0.149	-0.029
<i>Tichoderma</i> spp.	0.216	0
<i>Curvularia</i> spp.	-0.68*	-0.108
<i>Macrophomina</i> <i>phaseolina</i>	-0.494	-0.399
<i>Exserohilum</i> <i>pedicellatum</i>	0.064	0
<i>Thielavia</i> <i>terricola</i>	0	0.443*
<i>Fusarium</i> <i>oxysporum</i>	0.319	0.262
<i>Fusarium</i> <i>moniliforme</i>	-0.104	-0.36
<i>Fusarium</i> <i>chlamydosporum</i>	-0.12	0.329
<i>Fusarium</i> <i>equiseti</i>	-0.075	0.068

\*  $P < 0.05$

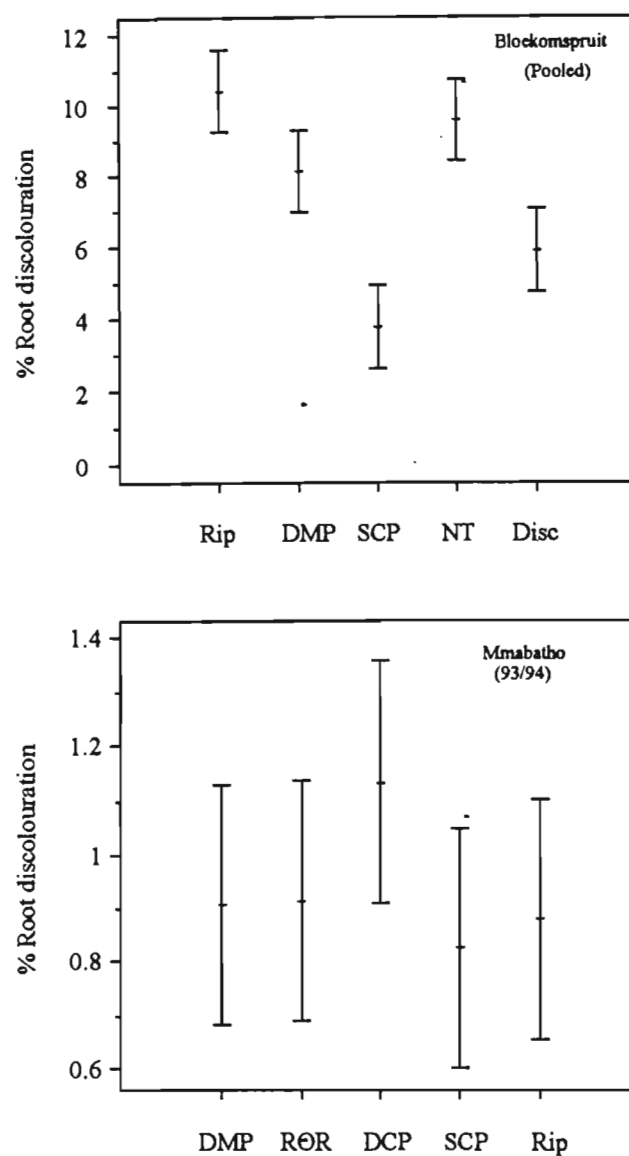


Fig. 1. Relationship between maize root discolouration and tillage treatments during the 1992/93 and 1993/94 seasons (pooled) at Bloekomspruit and during the 1993/94 season at Mmabatho.

Bars = LSD at  $P < 0.05$

(DMP=deep mouldboard plough, SCP=shallow chisel plough, NT=no-tillage, DCP=deep chisel plough, ROR=rip-on-row with lime applied in a band)

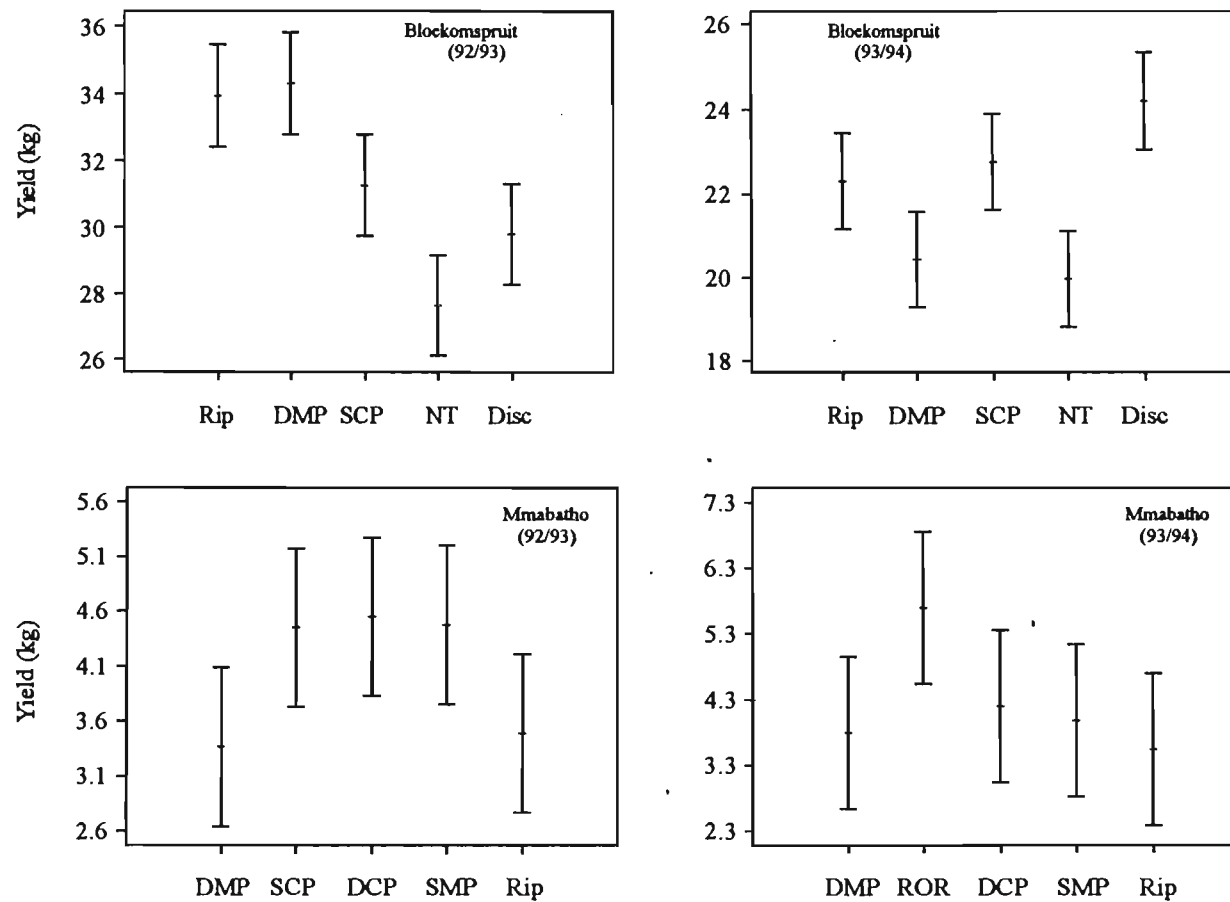


Fig. 2. Maize yield(kg) of tillage treatments over two seasons at Bloekomspruit and Mmabatho during the 1992/93 and 1993/94 seasons.  
Bars = LSD at  $P < 0.05$

(DMP= deep mouldboard plough, SCP=shallow chisel plough, SMP = shallow mouldboard plough, NT = no-tillage, ROR=rip-on-row with lime applied in a band, DCP=deep chisel plough)

## CHAPTER 5

# SPECTRUM OF ROOT-INFECTING FUNGI AND INCIDENCE OF MAIZE ROOT ROT IN CROP ROTATION SYSTEMS

### ABSTRACT

The effect of monoculture maize and crop rotation with maize, soybeans, sunflower and groundnuts were studied over two seasons. Isolation frequency of fungi involved in maize root rot was determined during both seasons. The spectrum involved differed slightly between seasons and may be ascribed to the difference in rainfall. The highest isolation frequency was obtained for *Phoma* spp. and *Fusarium* spp.. Crop rotation had a significant effect on the isolation frequency of *Thielavia terricola*, *Exserohilum pedicellatum*, *F. moniliforme* and *F. graminearum*. The effect on the isolation frequency of these fungi was, however, inconsistent. The influence of rotation systems appears to be complex. No single cropping system favoured all fungi and fungi were affected differently in various rotation systems. The isolation frequencies of only a few fungi correlated significantly with maize plant length, stover mass and maize yield. No correlation was recorded between isolation frequency and percentage root discolouration.

### INTRODUCTION

Root rot is known to cause severe problems in maize (*Zea mays* L.) cultivation, especially with continuous cropping of maize on sandy soils (Williams & Schmitthenner, 1963; Sumner & Bell, 1982; 1986; Scholte & s'Jacob, 1983; Sumner, Gascho, Johnson, Hook &

Threadgill, 1990). In South Africa maize is grown to a large extent in monoculture. Crop rotation may have distinct advantages when compared to monoculture maize in suppressing root rot inoculum in soil. Wheat, sunflower, sorghum and groundnuts are particularly suitable for rotation with maize and are generally planted as substitutes for maize in years with insufficient rain, before and during, the early planting season. Soybean also seems to be a suitable crop to precede maize, resulting in increased maize yield (Williams & Schmitthenner, 1963).

Root rot of maize is caused by parasitic soil fungi. *Pythium* spp. and *Fusarium* spp. are the main cause of maize root rot in the Netherlands (Hellinga, Bouwman, Scholte & s'Jacob, 1983; Scholte, 1987), while *P. graminicola*, Subr. *Rhizoctonia solani* Kühn and *R. zeae* Voorhees are important in the U.S.A. ( Rao, Schmitthenner, Caldwell & Ellet, 1978; Sumner & Bell, 1982; 1986). Scott (1982) considered *Phialophora zeicola* Deacon & Scott to be the major cause of maize root rot in South Africa, however, Chambers (1987a; b), indicated *Exserohilum pedicellatum* (Henry) Leonard & Suggs to occur more frequently than *Fusarium* spp. and *Trichoderma* spp..

The objective of this study was to determine the incidence of maize root rot and identify the major root infecting fungi in crop rotation systems with sunflower, soybeans, groundnuts and maize.

## MATERIALS AND METHODS

A field trial was conducted on a Clovelly soil (clay content of 8%) at Viljoenskroon in the north-western Orange Free State. The locality was selected on the basis of an ongoing long-term crop rotation trial. The rotation trial commenced during the 1989/90 season and was first monitored for root rot (present study) during the 1992/93 season. Maize root systems were sampled during the 1992/93 and 1993/94 seasons. Total rainfall of 386 mm and 769 mm were recorded during the two seasons, respectively.

Each plot where maize was planted, consisted of 14 rows, spaced 1.5 m apart and 50 m long. The trial consisted of seven treatments viz. monoculture maize (MMM), groundnuts-maize-maize (GMM), sunflower-maize-maize (SuMM), soybeans-maize-maize (SoMM), maize-sunflower-maize (MSuM), maize-soybeans-maize (MSoM) and maize-groundnuts-maize (MGM). The cultivars used were PAN6549 (maize), SNK37 (sunflower), Prima (soybeans), and Sellie (groundnuts) and treatments were replicated three times.

Fertilizer was applied in accordance to the recommendations of the FFSA-fertilizer manual. Trials were maintained until harvest by applying standard insect and weed control as required.

In the first season the length of 20 maize plants per plot was measured every alternate week (crown to tip of apical leaf) and stover dry mass was determined by drying the plant material at 60°C.



Sampling commenced four weeks after planting at fortnightly intervals until 50% tasselling. Five maize plants per plot, a total of 1050 plants for both seasons, were dug out, maintaining as much of the root system as possible. Roots were thoroughly washed in water to facilitate the removal of all soil and debris. Visual assessments of the percentage root rot were done during both seasons.

Discoloured root tissue (DR) were cut into 3 mm segments and surface-sterilised in 3.5% NaOCl for 1 min. After being rinsed twice in sterile distilled water, fifty root segments per sample were plated out onto each of Potato Dextrose Agar, Malt extract Agar and Potato Carrot Agar for identification. Isolated *Fusarium* spp. were cultured on Carnation Leaf Agar for identification. Cultures were incubated at 25°C for three days after which fungi isolated from maize roots were identified and quantified. Roots with no visible discolouration (CR) from the same sample were similarly treated in equal numbers and included as control treatments.

Yield was determined at plant maturity on the remaining plants and is presented as kg grain per plot.

Analysis of variance was done on isolation frequencies of fungi involved in maize root rot, stover mass, plant length and maize yield. The relationship between isolation frequency and stover mass, plant length, root discolouration and maize yield was determined using simple correlation analyses.

## RESULTS AND DISCUSSION

The spectrum of fungi isolated from maize roots at Viljoenskroon did not differ much between the two seasons (Table 1). Significant ( $P < 0.05$ ) differences in isolation frequency between the two seasons were obtained for *Alternaria* spp., *Thielavia terricola* (Gilman & Abbot) Emmons, *F. graminearum* Schwabe, *Trichoderma* spp., *Curvularia* spp., *F. oxysporum* Schlecht. emend. Snyder & Hans. and *F. moniliforme* Sheldon (Tables 1 and 2). These differences in isolation frequencies may be ascribed to the inter-seasonal rainfall differences. Williams & Schmithenner (1962) ascribed the crop x year interaction to variation in temperatures and rainfall and the resulting effect on residue decomposition which is higher in warm, wet periods. Sumner & Bell (1982) found the prevalence of *R. solani* AG-2 and severe brace root rot of maize in southwestern Georgia, USA, to be higher in a rotation system with maize and peanuts in irrigated fields than in other corn producing areas. Similar results were observed in this study with *F. oxysporum* that occurred more in the wetter season in MMM, GMM and MGM crop rotation systems (Table 1).

A significant ( $P < 0.05$ ) difference in isolation frequencies between DR and CR samples was recorded for *Trichoderma* spp., *Alternaria* spp., *E. pedicellatum*, *F. chlamydosporum* Wollenw. & Reinking and *F. equiseti* (Corda) Sacc.. *Trichoderma* spp. and *E. pedicellatum* may therefore, be regarded as lesion-causing organisms since they occurred more in discoloured root tissue. *E. pedicellatum* is a known maize root rot pathogen (Chambers, 1987a), but *Trichoderma* spp. are regarded as being slight to non-pathogenic and are isolated frequently throughout the season (Young & Kucharek, 1977; Sumner, *et al.*, 1990).

In this study, *Fusarium* spp. occurred the most in the MMM treatment during both seasons (Table 1). Significant differences ( $P < 0.05$ ) in isolation frequency of *F. moniliforme* and *F. graminearum* were observed between crop rotation treatments (Table 2). *F. moniliforme* had the highest incidence in the MMM, GMM, SuMM, and SoMM rotation treatments and the lowest where maize was rotated annually (MSuM, MSoM and MGM) (Fig. 1a). Incidence of *F. graminearum* was significantly ( $P < 0.05$ ) higher in the MMM and SoMM rotation systems (Table 2) than in the other crop rotation treatments. The crop rotation systems where maize was rotated with sunflower (SuMM & MSuM), however, decreased the isolation frequency of *F. graminearum* significantly ( $P < 0.05$ ) (Fig. 1b). *T. terricola* seemed to be associated with groundnut roots and was mostly isolated in rotation systems with groundnuts (GMM & MGM) and in the MSoM rotation system (Fig. 1c). The effect of crop rotation on the isolation frequency of *E. pedicellatum* was inconsistent. *E. pedicellatum* was significantly ( $P < 0.05$ ) higher isolated in the MMM system, whereas the isolation frequency decreased in SoMM, MSoM and MGM rotation systems (Fig. 1d).

The influence of rotation systems on maize root isolate populations appears to be complex. No single cropping system favoured all fungi, since fungi are affected differently by different rotation systems. Williams & Schmitthenner (1962) found considerably higher variation in the number of fungal species isolated from rotation plots, than from monoculture plots. They concluded that the variety of organic matter available for decomposition, is greater under rotation systems as opposed to monoculture, which may account for the higher variation in incidence of fungi in rotation systems. Some fungi, however, may adapt to a wide range of environmental conditions and crop rotation may therefore not be effective in reducing inoculum potential (Sumner & Bell, 1986).

Root rot severity has often been associated with root discolouration (Sumner & Bell, 1982; Hellinga, *et al.* 1983; Sumner, Dowler, Johnson, Chalfant, Phatak & Epperson, 1985). However, no significant differences between rotation systems with regard to percentage maize root discolouration were recorded in this study (Fig. 2). The incidence of fungi associated with maize roots did not significantly correlate with discolouration of maize roots. Scholte (1987), however, stated that a strong relationship between the incidence and severity of maize root rot and cropping frequency, exists. On the other hand, Blanquet, Van Schingen, Foucart, Maraite & Ledent, (1990) found no relation between maize root discolouration and number of years of maize monoculture.

Significant ( $P < 0.05$ ) differences were recorded between isolation frequencies of fungi and maize plant length, stover mass and crop rotation systems (Fig. 3). The highest stover mass and tallest maize plants were obtained in the SuMM rotation system. Scholte & s'Jacobs (1983) found the shortest plants in three successive cropping of maize, whereas the tallest plants were observed in two successive croppings of potato followed by one maize cropping. The only significant ( $P < 0.05$ ) correlation between maize plant length, stover mass of maize and isolation frequency of fungi was recorded for *T. terricola* (Table 3), but no obvious explanation could be found.

Soilborne diseases causing root and stalk rots resulted in yield decreases in maize monocropping systems in the United States and Europe (Williams & Schmitthenner, 1963; Sumner & Bell, 1982, 1986; Scholte, 1987; Lipps, 1988; Sumner, *et al.*, 1990). Local studies by Channon & Farina (1991) recorded yield decreases on sites in Natal which could not be ascribed to soil fertility or climatic constraints. The possibility, therefore, may exist

that a build-up of soilborne diseases may be responsible. However, little evidence was found in New Zealand of a build-up of maize root rot with successive crops of maize (Fowler, 1980). Although rotation practices resulted in significant ( $P < 0.05$ ) differences in maize yield in this study, these differences were inconsistent between the two seasons.

In the drier season (1992/93) the SuMM and SoMM systems gave the highest yield and the MSuM system the lowest (Fig. 4). There was a tendency for a higher maize yield in crop rotation systems with two successive maize cropping in the drier season, but the MSuM, MSoM and MGM practices showed a drastic increase in maize yield during the wetter season (1993/94). The isolation frequency of *Curvularia* spp. and *F. moniliforme* correlated significantly with yield in both seasons (Table 3). The positive correlation during the 1992/93 season and the negative correlation during the 1993/94 season with *F. moniliforme*, however, could not be explained.

The crop rotation trial monitored in this study started in the 1989/90 season, but maize roots were sampled only from the 1992/93 season. The variation in the incidence of fungi isolated from maize roots may be as a result of the ability of soilborne fungi isolated from maize roots to occur and survive on groundnuts, soybeans and sunflowers just as well as on maize roots. Another possibility that may exist is that these crop rotation systems may have a prolonged effect on maize roots and that the two seasons (1992\93 and 1993\94) monitored did not show the effect of crop rotation yet. Therefore, it is suggested that maize root systems be sampled for at least another season to conclude the effect of crop rotation on the incidence of fungi associated with maize roots.

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Table 1. Mean isolation frequencies of fungi occurring on maize roots in different crop rotation systems during the 1992/93 and 1993/94 seasons at Viljoenskroon

Fungi		Crop rotation system														
		MMM **		GMM		SuMM		SoMM		MSuM		MSoM		MGM		
		92/3	93/4	92/3	93/4	92/3	93/4	92/3	93/4	92/3	93/4	92/3	92/4	92/3	93/4	Mean
<i>Trichoderma</i> spp.	DR*	32	32	44	22	43	35	47	20	33	41	31	33	24	43	34
	CR	41	48	29	55	29	31	40	24	43	17	41	34	43	41	37
<i>Trichoderma</i> spp.	DR	7	28	10	27	6	24	2	32	2	26	6	38	8	11	16
	CR	5	11	7	21	7	18	1	16	8	12	2	14	5	6	10
<i>Verticillaria</i> spp	DR	0	0	1	0	0	0	1	0	0	0	3	0	1	0	0
	CR	5	0	2	0	2	0	1	0	1	0	3	0	6	0	1
<i>Fusovularia</i> spp.	DR	2	4	8	2	1	3	4	2	3	1	2	1	5	1	3
	CR	3	0	5	2	3	3	4	1	2	1	1	1	7	0	2
<i>Helicoverpia tricolora</i>	DR	4	0	7	0	3	0	5	0	4	0	12	0	9	0	3
	CR	1	0	12	0	3	0	3	0	3	0	12	0	15	0	4
<i>Macrophomina phaseolina</i>	DR	5	3	6	4	0	6	7	4	4	5	2	3	3	4	4
	CR	6	1	3	2	3	2	5	1	3	6	2	1	4	1	3
<i>Sclerotihilum dicellatum</i>	DR	6	4	5	5	6	5	1	1	2	3	7	1	1	4	4
	CR	5	1	4	0	4	0	1	0	3	1	0	0	0	0	1
<i>Fusarium oxysporum</i>	DR	10	45	12	46	13	31	16	25	6	39	9	35	23	42	25
	CR	9	38	14	29	9	20	18	21	8	38	9	26	9	29	20
<i>Fusarium niliforme</i>	DR	3	1	2	2	7	1	3	0	0	0	3	0	2	0	2
	CR	3	1	2	2	3	1	3	1	1	1	1	0	0	1	1
<i>Fusarium amydosporum</i>	DR	6	4	7	2	2	0	5	2	4	4	10	1	7	4	4
	CR	8	5	7	5	10	1	9	2	4	5	8	3	4	6	6
<i>Fusarium lutesi</i>	DR	23	20	11	16	13	12	17	18	8	24	21	9	8	12	15
	CR	27	28	14	23	25	13	27	22	9	27	15	13	12	16	19
<i>Fusarium minearum</i>	DR	6	0	3	0	1	0	7	0	0	0	8	0	4	0	2
	CR	5	0	5	0	1	0	6	0	2	0	2	0	0	0	2

Table 2. Indication of significant differences regarding the effect of crop rotation treatments, maize root discoloration (DR/CR), seasons (1992/93 and 1993/94) and the interactions involved regarding the crop rotation trial at Viljoenskroon

	<i>Phoma</i> spp.		<i>Trichoderma</i> spp.		<i>Alternaria</i> spp.		<i>Curvularia</i> spp.		<i>Thielavia</i> <i>terricola</i>		<i>Macrophomina</i> <i>phaseolina</i>	
	F *	P **	F	P	F	P	F	P	F	P	F	P
Treatment (a)	0.20	0.98	1.58	0.17	1.85	0.10	1.71	0.14	4.04	0.00	0.55	0.77
Discolouration (b)	1.00	0.33	7.93	0.01	3.84	0.06	0.69	0.42	1.12	0.30	2.97	0.09
Seasons (c)	0.42	0.52	66.29	0.00	51.66	0.00	8.00	0.01	233.21	0.00	918	0.35
Interactions:												
axb	1.09	0.38	0.64	0.70	1.26	0.29	0.47	0.83	0.75	0.62	0.39	0.88
axc	1.56	0.18	2.45	0.04	1.85	0.10	2.92	0.02	4.04	0.00	1.12	0.36
bxc	0.17	0.69	2.55	0.12	3.84	0.06	0.02	0.89	1.12	0.30	4.72	0.03

	<i>Exserohilum</i> <i>pedicellatum</i>		<i>Fusarium</i> <i>oxysporum</i>		<i>Fusarium</i> <i>moniliforme</i>		<i>Fusarium</i> <i>chlamydosporum</i>		<i>Fusarium</i> <i>equiseti</i>		<i>Fusarium</i> <i>graminearum</i>	
	F	P	F	P	F	P	F	P	F	P	F	P
Treatment (a)	2.30	0.05	0.48	0.82	4.41	0.00	1.53	0.19	1.74	0.13	2.22	0.05
Discolouration (b)	11.55	0.00	1.76	0.19	0.50	0.50	3.95	0.05	4.37	0.04	0.51	0.48
Seasons (c)	2.86	0.10	84.92	0.00	20.11	0.00	22.82	0.00	2.18	0.15	63.19	0.00
Interactions:												
axb	0.35	0.91	0.99	0.44	1.14	0.35	0.29	0.94	0.26	0.95	0.78	0.59
axc	0.59	0.74	1.88	0.10	2.32	0.04	1.82	0.11	2.60	0.03	2.22	0.05
bxc	5.22	0.03	0.60	0.45	5.73	0.02	0.42	0.53	0.00	0.96	0.51	0.48

\* F = Variance ratio

\*\* P = Probability level (P < 0.05)

Table 3. Correlation coefficients between isolation frequency of fungi and maize plant length, dry stover mass, root discolouration and maize yield for the 1992/93 and 1993/94 seasons respectively at Viljoenskroon

Fungi	Plant length (1992/93)	Stover mass (1992/93)	Discolouration (1992/93)	Discolouration (1993/94)	Yield (1992/93)	Yield (1993/94)
<i>Phoma</i> spp.	-0.12	-0.13	0.29	0.06	0.30	0.00
<i>Trichoderma</i> spp.	0.13	0.07	-0.04	0.23	-0.00	-0.30
<i>Curvularia</i> spp.	-0.05	0.11	-0.22	-0.08	0.07	-0.61*
<i>Alternaria</i> spp.	-0.41	-0.39	0.05		0.10	
<i>Thielavia terricola</i>	-0.61*	-0.54*	0.15		0.15	
<i>Macrophomina phaseolina</i>	-0.28	-0.09	0.24	0.16	-0.07	-0.03
<i>Exerohilum pedicellatum</i>	0.11	0.28	0.21	0.09	0.26	-0.04
<i>Fusarium oxysporum</i>	-0.13	0.05	-0.39	-0.1	0.17	-0.05
<i>Fusarium moniliforme</i>	0.1	0.52	0.05	-0.1	0.47*	-0.53*
<i>Fusarium clamydosporum</i>	-0.29	0.01	0.33	-0.06	0.13	0.03
<i>Fusarium equiseti</i>	0.06	0.07	-0.12	0.03	0.10	-0.31
<i>Fusarium graminearum</i>	-0.36	-0.16	-0.03		0.13	

\* Significance at  $P < 0.05$

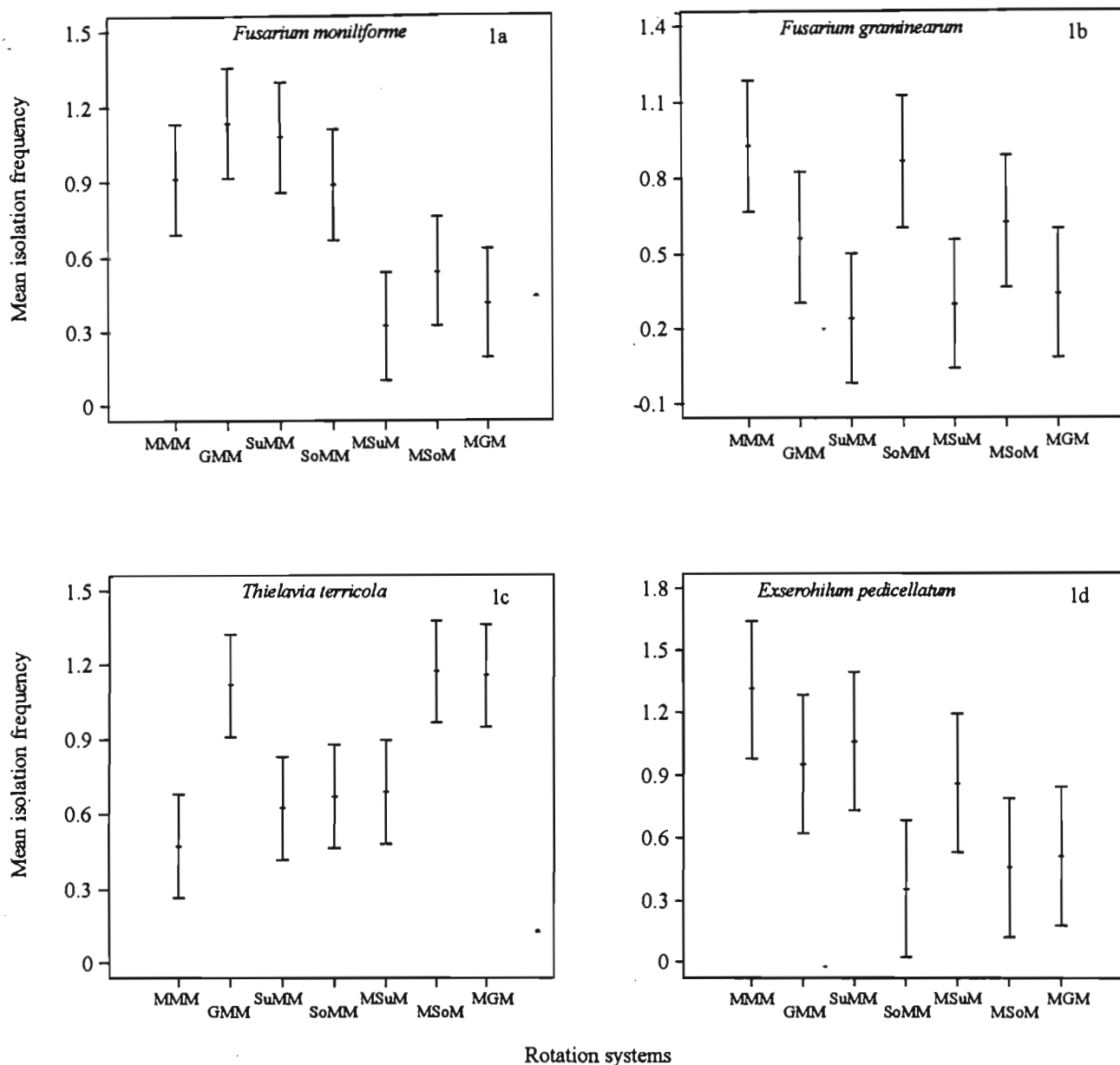


Fig. 1. The effect of crop rotation practices on the isolation frequency of fungi isolated from maize roots at Viljoenskroon.  
Bars = LSD at  $P < 0.05$

(MMM = Monoculture maize; GMM = Groundnuts-maize-maize; SuMM = Sunflower-maize-maize; SoMM = Soybean-maize-maize; MSuM = Maize-sunflower-maize; MSoM = Maize-soybean-maize; MGM = Maize-groundnuts-maize)

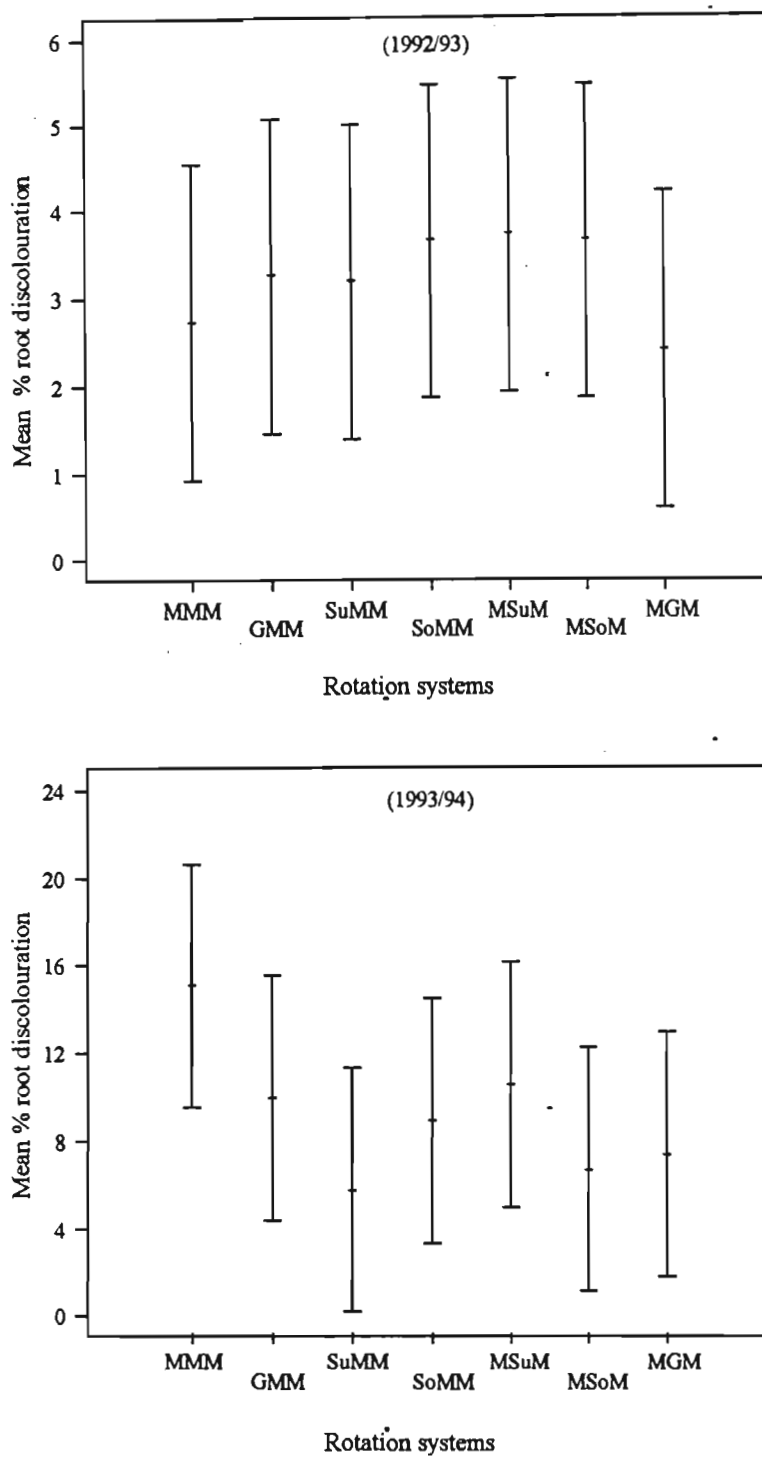


Fig. 2. The effect of crop rotation practices on maize root discoloration.  
Bars = LSD at  $P < 0.05$

(MMM = Monoculture maize; GMM = Groundnuts-maize-maize; SuMM = Sunflower-maize-maize; SoMM = Soybean-maize-maize; MSuM = Maize-sunflower-maize; MSoM = Maize-soybean-maize; MGM = Maize-groundnuts-maize)

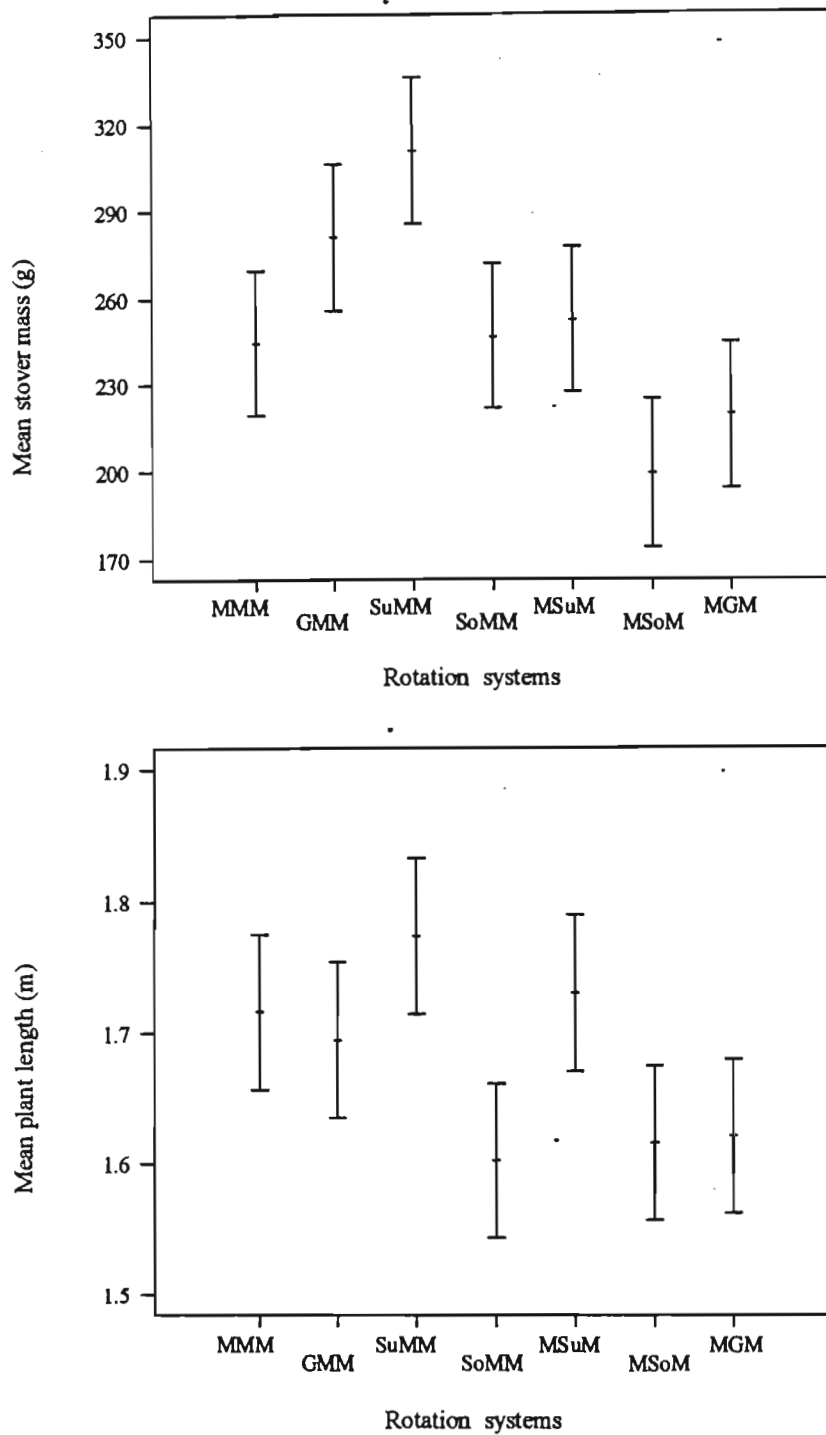


Fig. 3. The effect of crop rotation practices on maize stover mass and plant length during the 1992/93 season at Viljoenskroon.  
Bars = LSD at  $P < 0.05$

(MMM = Monoculture maize; GMM = Groundnuts-maize-maize; SuMM = Sunflower-maize-maize; SoMM = Soybean-maize-maize; MSuM = Maize-sunflower-maize; MSoM = Maize-soybean-maize; MGM = Maize-groundnuts-maize)

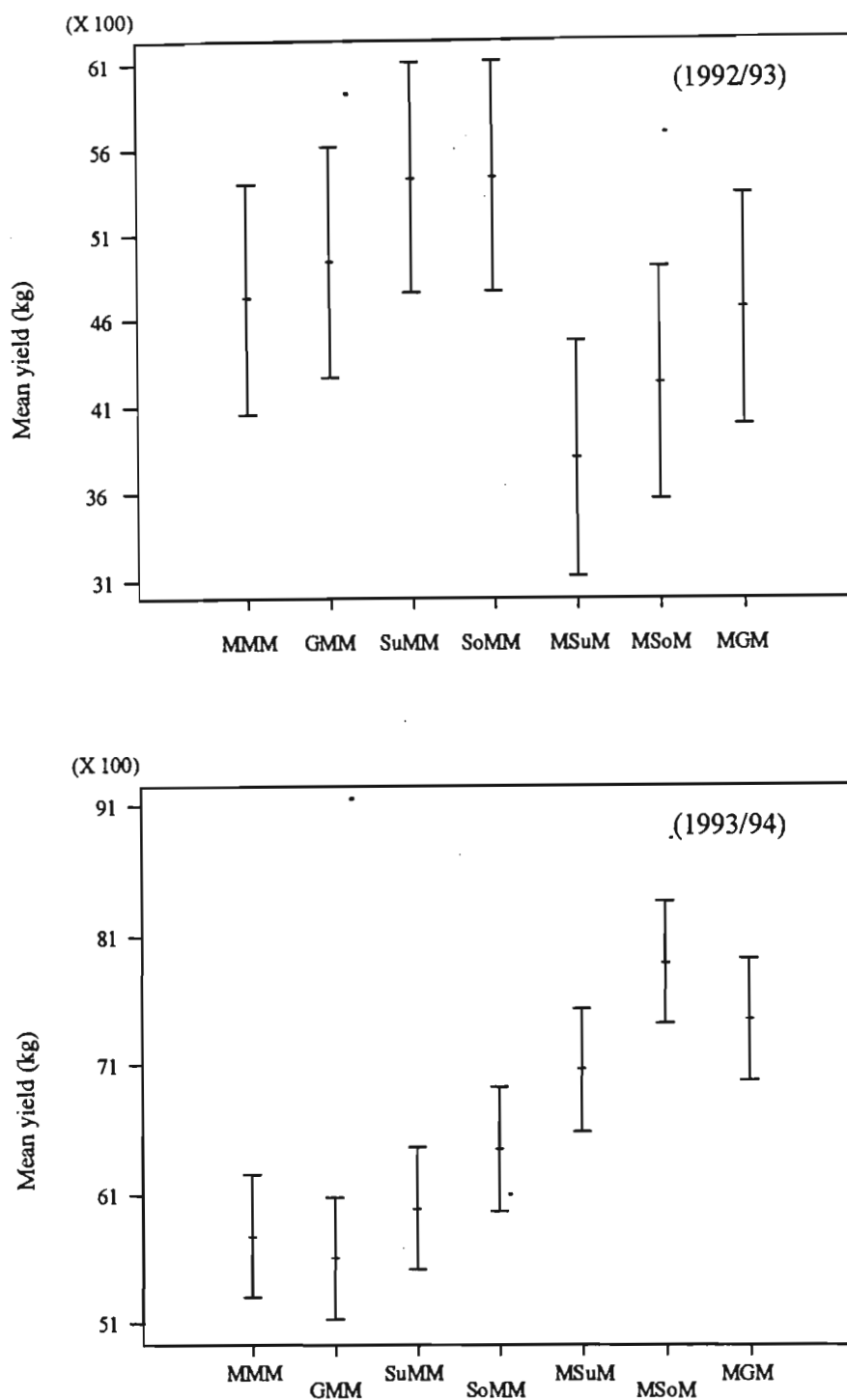


Fig. 4. The effect of crop rotation practices on maize yield for the 1992/93 and 1993/94 at Viljoenskroon.

Bars = LSD at  $P < 0.05$

(MMM = Monoculture maize; GMM = Groundnuts-maize-maize; SuMM = Sunflower-maize-maize; SoMM = Soybean-maize-maize; MSuM = Maize-sunflower-maize; MSoM = Maize-soybean-maize; MGM = Maize-groundnuts-maize)

## CHAPTER 6

### GENERAL DISCUSSION

Colonization of maize roots by fungi under natural conditions has not been studied to any significant extent because of the complexity of such investigations. Several fungal species, occurring in a complex of causal fungi separated in time and space are involved in maize root rot. Different fungal species occur throughout the season on roots, making it difficult to determine the primary pathogens. Furthermore, fungal species associated with maize roots differ between localities. Root rot, therefore, requires study at several localities in order to determine the spectrum of fungi involved. Differences in isolation frequencies of fungi from discoloured and clean root tissues were used to distinguish between fungi regarded as root pathogens and root colonizers. It is evident from the present study that *Exserohilum pedicellatum* (Henry) Leonard & Suggs and *Fusarium oxysporum* Schlecht. emend. Snyder & Hans. may be regarded as two of the most important fungi in the maize root rot complex in South Africa. Similar results were obtained by Chambers (1987a; b) regarding the pathogenicity of *E. pedicellatum*. Although contradictory results exist in relation to the pathogenicity of *F. oxysporum* (Hornby & Ullstrup, 1967; Palmer & Kommedahl, 1969; Scott, 1982; Moolman, 1992), it was as a predominant fungus at all localities. The contradictions regarding the pathogenicity of *F. oxysporum* could possibly be ascribed to the variation within the species. Leslie (1991) used a DNA-probing method to distinguish between various mating populations in *Gibberella fujikuroi* (Sawada) Wollenw. (*Fusarium* section Liseola). This method may be useful in determining between isolates of the more frequently isolated root-rot-inducing fungal species.



Numerous statements regarding "predisposition" of maize to root rot by stress factors have been made (Farley & Lockwood, 1964; Mortimore & Ward, 1964; Howell, 1966; Warren & Kommedahl, 1973; Fajemisin & Hooker, 1974; Ullstrup, 1977; Odvody & Dunkle, 1979). These, however, were never quantified. In the present study, however, fungi classified as root pathogens were more frequent in no stress and normal rainfall treatments than in the total stress treatment. Furthermore, these fungi had the highest isolation frequency early in the season in juvenile root tissue and declined as the tissue matured. These data, therefore, support the notion that these fungi are pathogens of actively growing plants, rather than stressed, senescing tissues (*sensu* Dodd, 1980).

Research to date has concentrated mainly on the effect of water stress on the root rot complex, but various other stress conditions may play a role, such as leaf and virus infections (Tu & Ford, 1971; Fajemisin & Hooker, 1974) and the presence of nematodes (Kisiel, Deubert & Zuckerman, 1969). These various stress conditions may have significant effects on root pathogens and it is therefore essential that future studies be conducted on these to quantify their effect on the root rot complex. Soil physical factors can affect maize root growth (Nowell & Wilhelm, 1987). Variation in soil factors are the result of tillage practices. Sumner & Bell (1986) found that tillage may influence plant residue distribution and soil compaction. Furthermore, they concluded that the effects of maize crown and brace root rot on yield reduction was greater if root growth is restricted by soil compaction. Different results were obtained regarding the various tillage practices studied in the present study. Environmental conditions, the presence of stubble on the ground and the extent of soil disturbance seemed to play an important role in the incidence of fungi in the maize root rot complex.

Yield decreases in monoculture maize in the USA and Europe have been ascribed to soilborne pathogens causing maize and stalk rot (Williams & Schmitthenner, 1963; Sumner & Bell, 1982; 1986; Scholte, 1987; Lipps, 1988; Sumner, Gascho, Johnson, Hook & Threadgill, 1990). In South Africa maize has traditionally been produced under a monoculture systems (Channon & Farina, 1991), although sorghum is sometimes grown in rotation with maize. The cropping of maize in a monoculture system in the present study has been shown to favour soilborne fungi which could lead to the build-up of inoculum potential in the soil.

A major shortcoming of root rot studies is quantification of the effect of disease on yield losses. Although root rot has been reported to cause severe yield losses (Le Roux, 1977; Sumner & Bell, 1986), contradicting reports reflect the complexity of the disease problem. This is compounded by the inability to standardize all other variables contributing to yield, while inducing a range of root rot severities. This could probably be done best under controlled conditions in a greenhouse, before verifying results under field conditions.

The spectrum of maize root rot fungi under South African conditions has been elucidated in the present study. Although many root colonizers were associated with maize roots, several pathogens have been identified. However, contradictory results regarding the pathogenicity of the fungi causing maize root rot, emphasizes the complexity of this disease. Furthermore, the isolation frequencies of fungi isolated from maize roots differed according to different water stress treatments, tillage practices and crop rotation systems. This information is essential if progress with the composition of an integrated disease control programme and the prevention of crop losses due to this disease is to be made. Although much has been

published with regard to maize root rot, it is obvious from the present study that many questions still remain unanswered.

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