

Chambers (1987) only used seven *F. graminearum* isolates in his study, compared to the 34 isolates that we tested. These isolates are currently being classified into the different lineages that have been identified within this fungus.



Fig. 2a. Reduction in growth of seedlings inoculated with *F. graminearum*. Left, control (uninoculated soil), right, inoculated soil.



Fig. 2b. Root rot caused by *F. graminearum*.
Seedling on left not inoculated (control)

Two isolates of *F. avenaceum* was evaluated for pathogenicity. Only one of the isolates was virulent (data not shown). *Fusarium avenaceum* is infrequently isolated from maize crowns and roots and this species does not seem to be an important component of the root rot complex. *Fusarium avenaceum* has been recorded as a virulent pathogen of crops such as canola (Calman, Tewari & Mugala, 1986), but only a weak pathogen of wheat roots (Lamprecht *et al.*, 1990). More isolates needs to be tested before any conclusions can be made about the importance of *F. avenaceum* as a soilborne pathogen of maize.

Fusarium equiseti did not cause any disease symptoms in the pathogenicity tests (Table 1). This fungus has been reported by Warren & Kommedahl (1973) in the USA and also elsewhere in South Africa by Smit *et al.* (1997), but it does not appear to be an important pathogen of maize.

Although *F. oxysporum* has been frequently isolated from maize crowns and roots in our field studies, this fungus seems to be a weak pathogen causing no significant reduction in seedling survival or growth and only occasional root rot symptoms (Table 1). *Fusarium oxysporum* was also frequently isolated from maize by other researchers in South Africa such as Du Toit (1968) and Chambers (1987a) and more recently by Smit *et al.* (1997) in Viljoenskroon. Smit *et al.* (1997) indicated that the fungus was isolated more frequently from from discoloured than clean root tissue. However, *F. oxysporum* is not regarded as an aggressive pathogen of maize. In pathogenicity tests, comparing the virulence of *F. oxysporum*, *F. verticillioides*, and *F. solani*, Soonthornpocet *et al.* (2000) reported *F. solani* and *F. verticillioides* (*F. moniliforme*) as pathogens, but not *F. oxysporum*. He showed that *F. verticillioides* (*F. moniliforme*) reduced the length of primary roots and *F. solani* reduced root weight of maize seedlings. The fungus has been listed as a wound pathogen of maize by Palmer & Kommedahl (1969) and Warren & Kommedahl (1973) concluded that *F. oxysporum* may function as a pathogen of maize roots when roots are wounded, other *Fusarium* spp. or fungi are part of the complex, or when temperatures are relatively high. In our study, *F. solani* and *F. verticillioides* caused very little root rot and only *F. verticillioides* caused some crown rot. None of these species reduced survival or growth of plants (Table 1). *Fusarium subglutinans*, *F. proliferatum*, *F. verticillioides* and *Fusarium* sp. 1 also caused both crown and root rot, but significantly less than *F. graminearum*, whereas no crown and root rot were recorded for *F. compactum*, *F. dimerum*, *F. dlamini*, *F. globosum*, *F. nygamai*, and *Fusarium* sp. 2. In our study we conducted the pathogenicity tests with single fungi, but may consider using pathogen complexes in future studies to investigate synergistic or protective effects among pathogenic fungi.

Phialophora spp. caused very distinct root rot when evaluated for pathogenicity on maize in this study (Fig. 3). *Phialophora* spp. also caused significant crown rot and significantly reduced growth of plants (Table 1). *Phialophora zeicola* was first isolated from maize roots and described by Deacon & Scott (1983) in South Africa, but there is limited information on the importance of this pathogen on maize. Deacon & Scott (1983) stated in their publication that *P. zeicola* predisposes maize to drought stress. They reported that the fungus caused little or no reduction in shoot growth and rated the fungus as a weak pathogen. In this study the fungus caused extensive root rot, even though we used a much lower inoculum concentration (0.5% w/w instead of 3% w/w) of the fungus than Deacon & Scott (1983). We isolated *Phialophora* spp. from maize roots for the first time in the 2007/2008 season. In both the 2007/2008 and 2008/2009 seasons *Phialophora* spp. were obtained only at the last sampling time (Lamprecht *et al.*, 2008, 2009). During the 2009/2010 season these fungi were obtained at both sampling times, but the incidences increased significantly from the first to the second sampling (Table 17, Facet 1). The isolation and identification of these fungi are very difficult and we are currently employing molecular techniques to correctly identify these fungi to species level.

Pyrenochaeta terrestris caused significant crown and root rot, but no reduction in survival and growth of plants in the pathogenicity studies that we conducted (Fig. 4, Table 1). This fungus is quite frequently isolated from maize crowns and roots in our field study (see Facet 1). Chambers (1987a) also previously reported the fungus on maize roots in South Africa, but he did not conduct pathogenicity studies with the pathogen (Chambers, 1987b). Smit (1997) obtained high numbers of *Phoma* spp. from maize roots, but it is uncertain whether these *Phoma* spp. included *P. terrestris*. We have obtained both *P. terrestris* and *Phoma* spp. from crowns and roots of maize in our field study (Facet 1), but it seems that the *Phoma* spp. are not as aggressive as the *P. terrestris* isolates. The *Phoma* spp. will also be identified to species level. *Pyrenochaeta terrestris* is regarded as the primary pathogen in the complex causing red root rot of maize (Mao *et al.*, 1998; Koenning *et al.* 2007). In Queensland, Australia, Ramsey (1990) reported *P. indica* to cause significant root rot. It is not certain at this stage whether *P. indica* occurs on maize in South Africa. Our isolates were morphologically identified as *P. terrestris*, but will be molecularly characterised in order to confirm the species of *Pyrenochaeta* that we used in our study. Red root rot occurs in many types of soil and the fungus survives well under a wide range of temperatures and pH conditions (White, 1999).



Fig 3. Root rot caused by an isolate of *Phialophora*.



Fig. 4. Root rot caused by *Pyrenochaeta terrestris*. Seedling on left not inoculated (control).

Pythium spp. isolated thus far in this study include *P. acanthicum*, *P. aristosporum*, *P. arrhenomanes*, *Pythium* HS-group, *P. irregulare*, *P. mamillatum*, *P. periilum*, *P. rostratifinges*, *P. spinosum*, *P. torulosum* and *P. ultimum* var. *ultimum*. Four of the species, *P. mamillatum*, *P. spinosum*, *P. torulosum* and *P. ultimum* significantly reduced survival, and *P. aristosporum*, *P. arrhenomanes*, *P. periilum*, *P. rostratifinges* and *P. torulosum* significantly reduced plant growth. Many *Pythium* species have been recorded to cause seedling blight and root rot of maize in other countries. These include *P. acanthicum*, *P. adhaerens*, *P. angustatum*, *P. aphanidermatum*, *P. arrhenomanes*, *P. dissotocum*, *graminicola*, *P. irregulare*, *P. myriotylum*, *P. paroecandrum*, *P. pulchrum*, *P. rostratum*, *P. splendens*, *P. tardicrescens*, *P. torulosum*, *P. spinosum*, *P. ultimum* and *P. vexans* (Rao *et al.*, 1978; Sumner *et al.*, 1985; Ramsey, 1990; Zhang *et al.* 1998; White, 1999). Recent studies indicate that *P. arrhenomanes* is the primary cause of root rot of maize in the Midwestern United States (Deep & Lipps, 1996). Ramsey (1990) reported *P. myriotylum* to cause significant root rot and in a study on *P. aphanidermatum*, *P. torulosum* and *P. graminicola*, Rao *et al.* (1978) showed that *P. graminicola* was the most aggressive. Van Zeeland *et al.* (1999) showed that *P. aristosporum* was the main cause of root browning and subsequent yield loss in a study in the Netherlands that was conducted to determine the causes of lower yields in continuous cropping of maize. Although *Pythium* spp. are considered major root pathogens in maize producing areas in certain parts of the United states and Europe (Rao *et al.*, 1978; Hellinga *et al.*, 1983) they have been considered to be of minor importance in South Africa (Du Toit, 1968; Kruger, 1970; Scott, 1982).

In our pathogenicity tests, *R. solani* AG-2-2 was the most virulent of the *Rhizoctonia* spp. and anastomosis groups tested, with the fungus causing reduction in survival and growth and significant crown and root rot (Fig.5a, b). In studies by Sumner & Bell (1982) they also reported *R. zae* less virulent than *R. solani* AG-2-2. The binucleate *Rhizoctonia* in their study were non-pathogenic. Pfahler & Petersen (2003) also reported AG-2-2 as a very virulent pathogen of maize. In our study the binucleate *Rhizotonia* AGs (AG-A, AG-F, AG-R) were very weak pathogens (Table 1).



Fig. 5a. Reduction in growth caused by *Rhizoctonia solani* AG-2-2. Left, uninoculated soil, right, soil inoculated with an isolate of *R. solani* AG-2-2.



Fig. 5b. Root rot caused by *Rhizoctonia solani* AG-2-2. Seedling on left not inoculated (control).

Stenocarpella maydis caused significant crown and root rot and significantly reduced the growth of plants (Fig. 6a, b, Table 1). These fungi are well known stalk and cob rot pathogens and one of the most important pathogens of maize in South Africa (Flett, McLaren & Wehner, 1998, White, 1999). Sutton & Waterston (1966) reported that *S. maydis* can infect roots and also cause seedling blight. *Stenocarpella maydis* appears to be quite important during the later stages of the season when we isolate the fungus from both crowns and roots. During the 2007/2008 season incidences of *Diplodia/Stenocarpella* spp. were significantly positively correlated with crown rot severity and significantly negatively correlated with grain yield (Lamprecht *et al.*, 2008).



Fig. 6a. Growth of plants in soil inoculated with an isolate of *Stenocarpella maydis*. Left, uninoculated soil, right soil inoculated with the fungus.



Fig. 6b. Root rot caused by *Stenocarpella maydis*.
Seedling on left not inoculated (control)

Trichoderma spp. are very frequently isolated in our field study (see Facet 1). All the *Trichoderma* spp. obtained by us during the course of our field study were tested for pathogenicity, since McFadden & Sutton (1975) reported *T. koningii*, *T. harzianum* and *T. hamatum* to produce first internode lesions in maize seedlings. Two of the species, *T. asperellum* and *T. dorotheae* caused significant reduction of survival compared to the control. Although we observed discolouration of subcrown internodes of maize seedlings inoculated with the *Trichoderma* spp., no crown or root rot were observed (Table 1). All the *Trichoderma* species, except for *T. asperellum* caused a significant increase in growth of plants compared to the control (Table 1). *Trichoderma* spp. are the second dominant fungi isolated from crowns and roots of maize in our field trials. During the initial stages of our field studies, plants were sampled three times during the season and it was clear that *Trichoderma* was significantly more frequently isolated in the beginning than at the end of the season. There are many reports on the biocontrol effects of *Trichoderma* spp on diseases of maize. It has also been reported that *T. harzianum* can induce resistance in maize plants and can result in growth promotion of plants (Windham *et al.*, 1989; Harman *et al.*, 2004; Harman, 2006; Sobowale *et al.*, 2007). It would be useful to evaluate the biocontrol effect of *Trichoderma* spp. on some of the most important pathogens obtained in our study.

With regard to the unidentified fungi that caused crown and root rot and reductions in survival and growth, these fungi are currently being identified molecularly. The Unidentified 7 fungus were quite often isolated at the end of the season and may be an important role player in the crown and root rot complex.

The inoculum level (0.5% w/w) used by us in our pathogenicity trials was much lower than that used by other researchers. Kruger & Ragdaki-Papadaki (1979) used a 2–3% w/w (inoculum/growth medium) inoculum ratio, Chambers *et al.* (1987) a 1, 2 and 3% w/w and Deacon & Scott a 3% w/w ratio. Despite this difference, we obtained good results in that we were still able to rate root rot severity for pathogens that caused a significant reduction in survival such as *F. graminearum* and *R. solani* AG-2-2. We were also able to differentiate between the more virulent pathogens such as *F. graminearum*, *Phialophora* spp. and *R. solani* AG-2-2 and *S. maydis*.

Summary of pathogenicity tests

- Of the fungi frequently isolated:
 - a) *F. graminearum*, *T. asperellum*, and *T. dorotheae* significantly reduced survival of seedlings.
 - b) *F. graminearum*, *Phialophora* spp, *P. terrestris* and *S. maydis* caused significant root rot.
 - c) *F. graminearum*, *F. proliferatum*, *F. subglutinans*, *Phialophora* spp., *P. terrestris* and *S. maydis* caused significant crown rot.
 - d) *F. graminearum*, *Phialophora* spp. and *S. maydis* significantly reduced growth.
- All the *Trichoderma* species, other than *T. asperellum*, significantly increased plant growth.

CONCLUSIONS

Results obtained from the pathogenicity tests showed that a large number of fungi isolated from diseased maize crowns and roots can cause crown and root rot and reduce survival and growth of maize plants. Of the fungi most frequently isolated from diseased maize in our trial conducted for Facet 1, *F. graminearum*, *Phialophora* spp., *P. terrestris* and *S. maydis* proved to be the most aggressive pathogens on maize seedlings. The pathogenicity tests, however, need to be repeated to confirm results obtained this year and to comply with requirements for publication.

The inoculum level used in our studies was much lower (0.5%) than that normally (3%) used by researchers in pathogenicity tests on maize. We reduced the inoculum level in order to enhance survival, particularly after inoculation with aggressive pathogens, so that we were able to rate root rot severity and crown rot incidence. In spite of this low inoculum level the aggressive pathogens, such as *F. graminearum*, caused a significant reduction in survival. A high inoculum level of the aggressive pathogens would have resulted in no survival.

In the pathogenicity test reported here, single isolates were used for inoculation. Under field conditions, a complex of organisms is always associated with diseased

maize crowns and roots, and our pathogenicity tests showed that a large number of fungi can cause crown and root rot. It is therefore important to evaluate the effect of combinations of the different pathogens on survival, growth reduction, crown and root rot of maize. Isolations done for Facet 1 showed clearly that a succession of organisms are associated with maize plants at different times during the growing season (Lamprecht *et al.*, 2007, 2008). It, therefore, seems logical that certain pathogens will infect seedlings, while other pathogens are more important later in the season. The fact that *Phialophora* spp. and *S. maydis* are more frequently isolated at the end of the season may be an indication that these pathogens are more important on older plants. To demonstrate the succession of organisms in a glasshouse will however be very difficult.

Crop rotation is often recommended to manage soilborne plant diseases. In our field trial, it is clear that crop rotation can significantly reduce crown and root rot. The effect is often more significant early in the season than later (see Facet 1). Since crop rotation can also have benefits other than disease control, it is important to conduct cross-pathogenicity tests with the most important soilborne pathogens identified in this study, on rotation crops such as black oat, canola, crambe, stooling rye and wheat. This is essential to determine the potential of these crops to act as hosts of pathogens that are involved in soilborne diseases of maize.

Our results showed that the *Trichoderma* spp. do not cause crown or root rot. *Trichoderma asperellum* and *T. dorotheae* caused a significant reduction in survival of seedlings and this needs to be confirmed in a follow up trial. An interesting result was the significant increase in growth of plants inoculated with the different *Trichoderma* species. It would be valuable to determine whether the *Trichoderma* spp. could protect maize seedlings against infection by the most aggressive pathogens. Research results obtained for Facet 1 (Lamprecht *et al.*, 2006, 207 2008) showed a high incidence of *Trichoderma* spp. in the beginning of the season and a significant reduction of these fungi towards the end of the season. If involved in biological control, it therefore seems that the resident *Trichoderma* spp. may have a more pronounced effect on pathogens in the beginning than the end of the season.

Although some of the unidentified fungi are not frequently isolated from diseased maize crowns and roots, it is important to determine their identities. One of these, Unidentified 7, often encountered during the later stages of the season, is currently molecularly characterised.

The pathogenicity study conducted by us is to date the most comprehensive study of this nature on soilborne diseases of maize in South Africa. All fungi associated with diseased crowns and roots of maize were evaluated for their ability to cause crown and root rot and a reduction in survival and growth. In order to compare our results with those of researchers in other countries, the identity of all fungi used in these tests is being molecularly confirmed. The pathogenicity tests conducted in 2010 will have to be repeated and expanded in 2011 to include combinations of pathogens and cross-pathogenicity on rotation crops.

There is a need now to examine the effects of cover and factors probably associated with it (earthworm activity, topsoil moisture content, infiltration-rate, treatment and nutrition) on final grain yield.

Actual grain yields and relative yield values for the 2008/2009 and 2009/2010 seasons are presented in Table 29. These two seasons were not markedly different, other than in terms of when moisture stress occurred (March in 2009 and February in 2010) and it will be noted that in spite of the fact that mean yields in the two seasons differed by over 1200 kg/ha, the relative response to this extra cover was remarkably similar. Also, in the 2006/2007 season, prior to the specific introduction of plus- and minus-cover treatments, the benefit of a soya-wheat-maize treatment over the soya-fallow-maize treatment was approximately 8% (Table 13). This may well be largely fortuitous, but, nevertheless, clearly illustrates the effects of cover in “average” seasons. The effects of cover on relative yield are unusually consistent for across season field derived data. There can be no doubt that the data provide an excellent first approximation of the contribution of good cover in the soil-bioclimatic group in which they were obtained. They will, however, probably be less in abnormally favourable conditions (Lamprecht *et al.*, 2008) or greater in harsher stress conditions. Here, yet again, the poor performance of the FM treatment is conspicuous and cannot be ignored. Surprisingly, it has not even proved superior to the bare fallow, no-till treatment.

As previously noted, the much smaller and non-significant effect of cover on yield in the anhydrous ammonia treated plots may well be due to the fungicidal properties of this product. This effect has been evident in all previous seasons (Lamprecht *et al.*, 2007, 2008 and 2009) and will be discussed further in the section below.

During the 2008/2009 season, there were indications that marginal K nutrition in minus-cover plots could possibly have played a role in depressing yields in these treatments (Lamprecht *et al.*, 2009) and for this reason the K status of all plots was substantially increased and minus-cover plots received further applications of K equivalent to the quantity returned in wheat straw to plus-cover plots (see Materials & Methods). It is apparent in Tables 14, 15 and 16 that some evidence of differential K status continues to exist, but, importantly, soil and leaf data (Tables 14 & 16) conclusively show that the levels were more than adequate in all treatments. This effectively excludes the possible role of nutritional effects, other than those of anhydrous ammonia on Mn uptake, from any involvement in the yield responses discussed here.

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