Table 28 provides yield comparisons 64 DAP, earthworm counts four days prior to this, and temperature and moisture readings nine days after the yield assessment (the crown and root rot assessments 64 DAP will be discussed separately below). Here, there is an indication that yield differences in the favour of plus-cover plots were starting to emerge. Similarly, soil temperatures were consistently, albeit not significantly, lower in these treatments and soil moisture contents were markedly higher. Earthworm count differences were also very marked and significant or nearly so in all but the methyl bromide treatments. Clearly, extra cover was promoting earthworm activity and either reducing evaporative water loss or enhancing rainfall infiltration through increased macroporosity. The beneficial effects in the case of maize fallow plots (FM & FP) are particularly noteworthy, as in this comparison the minus-extra-cover plots (FM) had a biomass load (approximately 9000 kg/ha), which would normally be considered more than adequate in no-till cropping systems.

Table 28. **Wheat straw cover effects on relative yield, crown and root rot severity ratings 64 DAP, earthworm counts 60 DAP, and soil temperature and moisture content 73 DAP.**

TREATMENT♦	RELATIVE YIELD (%)	SOIL TEMPERATURE (° C)	SOIL MOISTURE (%)	WORM COUNT (/m²)	CROWN ROT SEVERITY	ROOT ROT SEVERITY
ANM	97	27.4	6.0	41	0.50	1.42
ANP	100	25.9	12.6	75	0.71	1.75
BF	80	26.6	11.5	7	1.17	2.33
FM	83	25.9	9.9	21	0.88	1.83
FP	88	25.7	16.6	67	0.83	2.00
MBM	90	26.4	5.8	9	0.50	1.33
MBP	96	25.8	12.7	35	0.63	1.63
TM	86	26.2	5.5	17	0.79	1.79
TP	94	25.6	12.8	83	0.75	1.71
C	86	26.1	15.4	103	1.25	2.33
LSD (0.05)	12	2.5	3.6	44	0.23	0.41

♦ **See Table 2 for description of treatments.**

M = minus extra cover.
P = plus extra cover.

Table 29. **Wheat straw cover effects on relative yield, crown and root rot severity ratings, and root lodging 140 DAP, and on earthworm counts, soil temperature and moisture content 135 DAP, and infiltration rate 154 DAP.**

TREAT-MENT♦	RELATIVE YIELD (%)	SOIL TEMPERA-TURE (° C)	SOIL MOISTURE (%)	WORM COUNT (/m²)	CROWN ROT SEVERITY	ROOT ROT SEVERITY	ROOT LODGING (PLANTS/ PLOT)	INFILTRA-TION RATE (MINS/20L)
ANM	80	26.0	14.3	7	1.58	2.71	67	5.07
ANP	86	24.0	20.7	14	1.63	2.79	20	1.82
BF	69	25.0	15.0	0	2.88	3.54	57	33.37
FM	63	24.0	18.7	17	1.88	3.00	29	18.51
FP	83	24.0	26.0	43	2.17	3.13	11	6.01
MBM	64	25.9	12.9	3	2.30	3.13	176	10.47
MBP	93	23.1	17.3	19	1.42	2.42	78	5.56
TM	62	26.0	9.6	9	2.75	3.50	177	5.45
TP	90	23.9	19.7	12	1.96	2.88	95	1.77
C	98	24.0	24.6	45	1.79	2.92	7	4.62
LSD (0.05)	10	2.3	4.6	19	0.51	0.29	29	6.3

♦ **See Table 2 for description of treatments.**

M = minus extra cover.

P = plus extra cover.

Table 29 provides information obtained at a later stage; earthworm counts and temperature and moisture determinations obtained five days prior to plant sampling and infiltration rate data collected some two weeks after sampling. At this stage the yield benefits of extra cover were highly significant in the rotovated and fallow plots, significant or nearly significant temperature benefits were evident other than in the maize fallow comparison, and in all cases the benefits of cover in terms of moisture content were clearly evident. Similar trends were evident in the case of earthworm counts and, here again, the effect in maize fallow plots deserves to be emphasised.

Infiltration-rate data presented in Table 29, although only statistically different in the case of maize fallow plots – high coefficients of variability are associated with such determinations – clearly tend to support the hypothesis that cover fostered earthworm activity and that this resulted in increased macroporosity and enhanced rainfall entrapment in the soil profile. This is not unexpected (Triplett & Dick, 2008).

However, it was surprising that rotovated and ripped plots proved to have infiltration times equally rapid to the no-till treatments with cover, in spite of lower earthworm counts (Fig. 10). Only the FM and BF no-till treatments were significantly more poorly drained. Govaerts *et al.*, (2006) made similar observations on volcanic soils in Mexico, but there are numerous reports to indicate that tillage may have the opposite effect on many soils (Bradford & Peterson, 2000), due to tillage disrupting the macropores created by earthworms, roots and, in some cases, natural cracks. It seems possible, therefore, that as was speculated to be the case in the Mexican study, the use of a single ring (the 0.25 m² earthworm confinement ring) resulted in appreciable lateral water flow where the soil had been tilled. Data to be obtained next season, when infiltration-rate measurements are to be made the day after the soil is saturated with the equivalent of 80 mm of rainfall (the quantity of vermifuge used for earthworm counts) should clarify the situation. Nevertheless, it is again evident that the benefits of normal maize fallow (FM), even with a relatively heavy stover load, warrant consideration. The bare fallow planted no-till had a particularly poor infiltration-rate and situations close to this are not uncommon among no-till practitioners (Fig. 9)

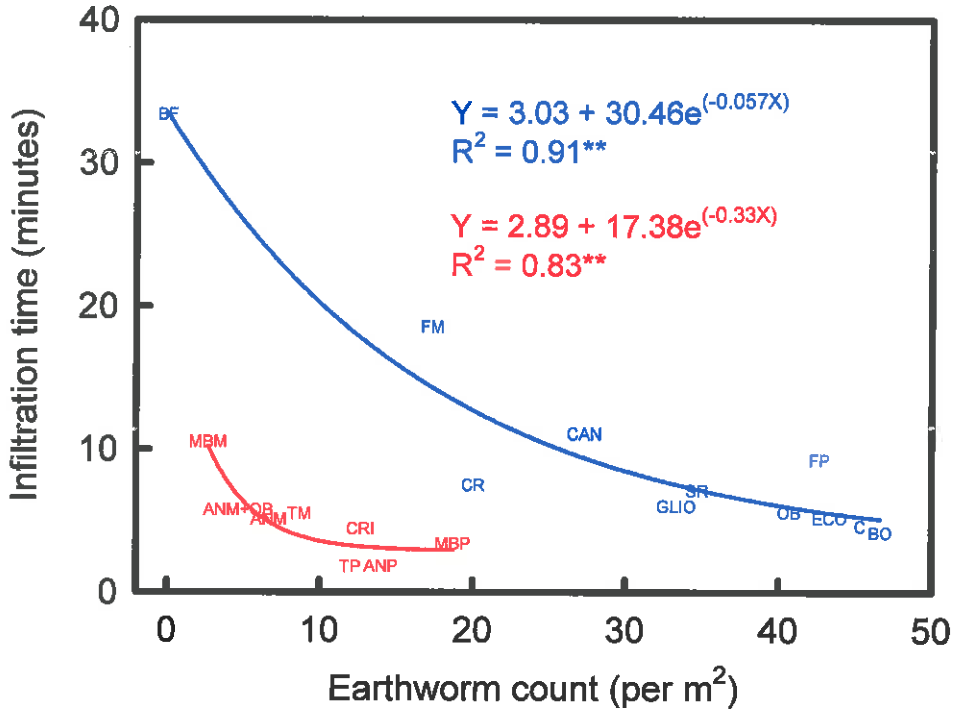

Fig. 10. **The effect of earthworm numbers 135 DAP on infiltration-rate determined 154 DAP (no-till treatments in blue, tilled treatments in red).**

The above discussion explains why, in our study, the soil moisture content of the surface 0-60 mm appears to be a more reliable measure of the moisture-related benefits of cover. Fig. 11 shows the relationship between cover (earthworm counts) and soil moisture content and there is clearly a very satisfactory correlation, especially if the fungicidal effects of anhydrous ammonia are taken into account.

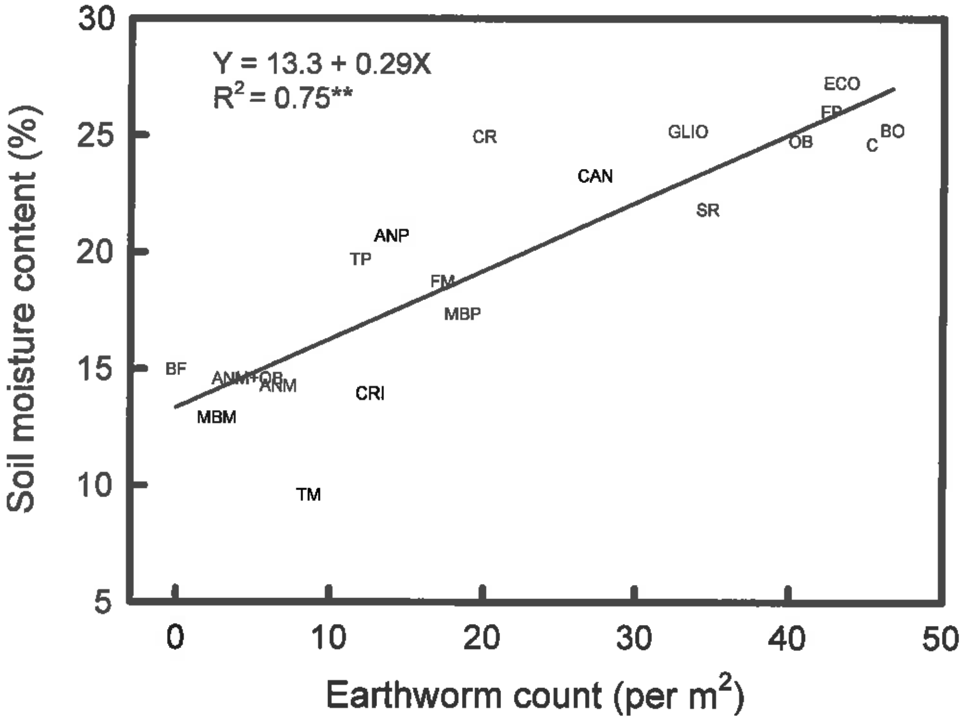

Fig. 11.The effect of earthworm numbers on soil moisture content 135 DAP.

Finally, there is a need to briefly examine the effects of cover on root lodging. From the data presented in Table 12 it is clear that the incidence of lodging was very substantially reduced by the presence of extra cover. It is not yet clear what the causal mechanism was, but it seems probable that reduced moisture stress was intimately involved (Fig. 12). Perhaps this ameliorated the effects of soilborne root diseases? This will be discussed in more detail in the sections below.

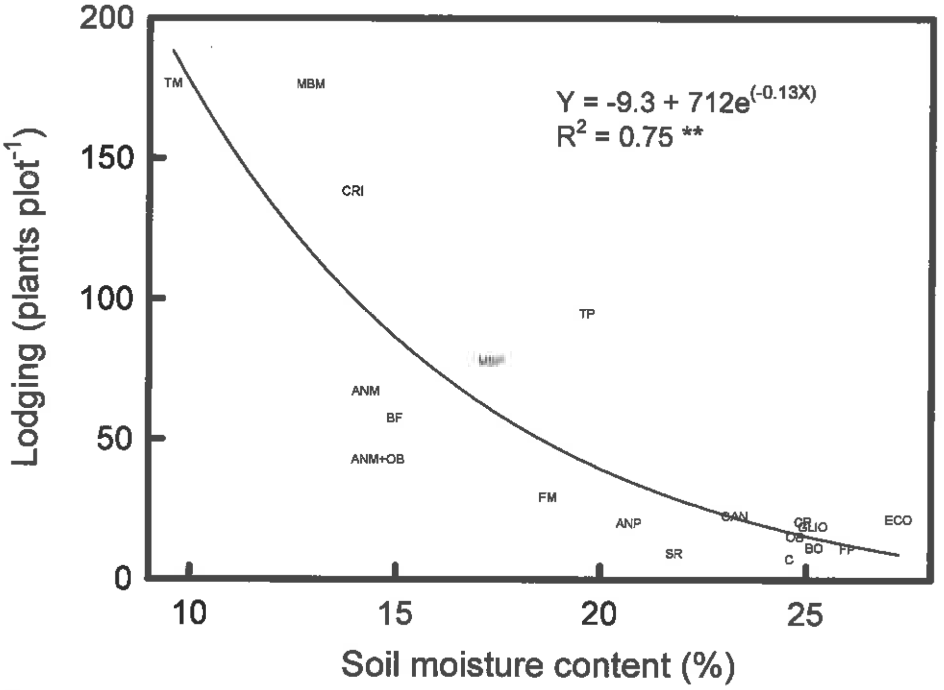

Fig. 12 The effect of topsoil (0-60 mm) moisture content 135 DAP on root lodging 140 DAP.

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 30. 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.

Table 30. **Cover effects on grain yield over the past two seasons.**

TREATMENT ♦	**GRAIN YIELD (kg/ha)**		**RELATIVE YIELD (%)**	
	2008/2009	**2009/2010**	**2008/2009**	**2009/2010**
ANM	13116	15450	94	99
ANP	13980	15650	100	100
BF	11342	12453	81	80
FM	11654	12637	83	81
FP	12733	14112	91	90
MBM	11810	13129	84	84
MBP	13272	14640	95	94
TM	11711	12783	84	82
TP	13187	14215	94	91
C	13471	14066	96	90
LSD (0.05)	1084	802	8	5

♦ **See Table 2 for description of treatments.**
M = minus extra cover.
P = plus extra cover.

Crown and root rots

Comparison of these treatments in terms of crown and root rot severity indicated that the effects 64 DAP (Table 10) were non-significant. However, 140 DAP (Table 11) the situation had changed quite markedly. While the benefit in the fallow (FM & FP) and anhydrous ammonia treated plots did not reach statistical significance, that recorded in tilled (TM & TP) and methyl bromide fumigated plots (MBM & MBP) was large and highly significant. In fact, the MBP treatment had the lowest root rot severity of all treatments in the trial, while the MBM treatment only ranked 14th (Fig. 13). The addition of cover to the methyl bromide plots reduced root rot severity by 22.6% and to the tilled plots by 17.8%. The relative yield benefit was 6% and 8%, respectively.

(a)

Roots of maize after treatment with methyl bromide plus extra straw cover (MBP)

(b)

Roots of maize after treatment with methyl bromide minus extra straw envelop (ANP).

Fig 13. **Roots of maize 140 DAP after treatment with (a) methyl bromide plus extra straw cover (MBP) and (b) methyl bromide minus extra straw cover (MBM). At this stage the relative yields were 93% and 64%, respectively.**

Fungi associated with crowns and roots

Application of extra straw did not significantly increase the incidences of *F. graminearum* in plant roots (Table 17). During the previous season the application of straw only significantly affected the incidences of *F. graminearum* in the fallow treatment, where the wheat straw cover significantly increased the incidences of *F. graminearum* for the FP compared to the FM treatment. The lowest incidences of *F. graminearum* in roots were again recorded for the bare fallow (BF) treatment. Results on incidences in crowns showed a significantly higher incidence of the fungus in crowns at the second sampling time for the TM treatment compared to the TP as well as the other treatments. As already mentioned it is difficult to explain the high incidence of the fungus in crowns of maize subjected to this treatment.

Extra wheat straw cover did not significantly affect the incidence of *F. oxysporum* in crowns and roots (Table 17). During the previous season significantly higher incidences of *F. oxysporum* were recorded in roots of the ANP compared to the ANM treatment. During the 2008 season incidences of *F. oxysporum* were significantly increased by the application of straw in all treatments, but during the 2006/2007 season incidences of *F. oxysporum* was decreased with full cover (Lamprecht *et al.*, 2007, 2008, 2009). According to Smit & McLaren (1997) results that they obtained suggested that the absence of water stress may promote root colonization by *Exserohilum pedicellatum*, *F. equiseti*, *F. verticillioides* and *F. oxysporum*.

Similar to the previous season, incidences of *Pyrenochaeta terrestris* in crowns and roots were not significantly affected by the application of extra wheat cover (Table 17). Results from the 2007/2008 season showed a significant decrease in the incidences of the fungus in roots of the methyl bromide and tillage treatments when cover was applied. Unfortunately the same results were not obtained during this season and the 2008/2009 season. Application of cover also did not significantly affect the incidences of *Pythium* spp. in roots (Table 17).

Similar to the previous season, the highest incidences of *Trichoderma* spp. were recorded for the MBM and MBP treatments, which is most probably because of a lack of competition for colonization of fumigated soil by other organisms. Although the application of straw cover reduced the incidences of these fungi in the roots, these reductions were not significant and the application of wheat straw cover did not significantly affected the incidences of the fungi in roots. For the crowns a significantly higher incidence of these fungi were recorded for the MBM than the MBP treatment, but the FP and FM, ANM and ANP, and TM and TP treatments did not differ significantly with regard to *Trichoderma* spp isolated from the crowns. During the 2008 season the application of straw cover only significantly reduced the incidences of *Trichoderma* spp. in roots of the anhydrous ammonia treatment.

Similar to the previous season, there were no significant differences in the incidences of *Diplodia/Stenocarpella* spp. in crowns and roots of maize from treatments with and without extra straw cover. During the previous season, in all instances, except for the TM and TP treatments, there were higher incidences of the *Diplodia/Stenocarpella* spp. recorded for the treatments without than those with cover, and the highest incidences for the bare fallow (BF) treatment where the maize stubble from the previous season was also removed.

According to Govaerts *et al.* (2008) zero-till with residue removal is an unsustainable practice since it decreased populations of beneficial organisms such as fluorescent *Pseudomonas* spp.

Nematodes

Comparison of the cover treatments in terms of the number of herbivores and beneficial nematodes indicated that the effects 64 DAP were small (Table 8). The anhydrous and tilled treatments (ANP, FP) with cover had higher population numbers of herbivores than the treatments without cover (ANM, FM). During the previous two seasons higher herbivore population numbers were observed in the fallow, methyl bromide and tilled treatments (FP, MBP, TP) (Lamprecht *et al.*, 2008: 2009). A possible reason for the consistency in the fallow plus cover treatment supporting higher nematode numbers is that the soil temperature is lowered to the optimum temperature for nematodes. The optimum temperature required for nematode development is often correlated to the optimum temperature required for good plant growth (McDonald & Nicol, 2005). According to Raaijmakers *et al.* (2009) plant residues left on or near the soil surface may play a role to increase disease suppresiveness through the promotion of general microbial activity, but it may also in some cases not only promote microbial activity but may also help to preserve the pathogens, preventing a decrease of inoculum density. In an Australian study Rahman *et al.* (2007) reported that stubble retention leads to high population numbers of bacteriophagous (primarily Rhabditidae) and omnivorous nematodes.

Microbial diversity and activity in soil

Although statistically significant difference could be observed between the number of different bacterial species (Shannon Index), no significant differences could be observed in the abundance of bacterial species within soil microbial communities (Evenness Index) within cover treatments at 64 and 140 DAP (Table 31).

Table 31. **Diversity Indices of soil microbial populations in wheat straw cover treatments 64 and 140 DAP.**

Cover Treatment	Shannon (H') 64 DAP ^{z}	Shannon (H') 140 DAP ^{z}	Evenness (E) 64 DAP ^{z}	Evenness (E) 140 DAP ^{z}
ANM	2.680^{cd}	2.525^{abc}	0.836^{a}	0.839^{a}
ANP	2.728^{cd}	2.671^{bcd}	0.840^{a}	0.846^{a}
BF	2.687^{cd}	2.360^{ab}	0.852^{a}	0.794^{a}
FM	2.875^{d}	2.492^{abc}	0.845^{a}	0.848^{a}
FP	2.590^{bcd}	2.668^{bcd}	0.847^{a}	0.843^{a}
MBM	2.580^{bcd}	2.250^{a}	0.855^{a}	0.831^{a}
MBP	2.734^{cd}	2.748^{cd}	0.847^{a}	0.842^{a}
TM	2.769^{cd}	2.543^{abc}	0.827^{a}	0.839^{a}
TP	2.659^{bcd}	2.754^{cd}	0.850^{a}	0.853^{a}

^{z}Means within a column followed by the same letter do not differ significantly (P = 0.05).

Significant reduction in the number of different bacterial species within the microbial population (Shannon Index) could be observed in bare fallow (BF) plots, fallow plots without added wheat straw (FM) and methyl bromide treated plots without added wheat straw (MBM) (Table 31). This could be attributed to the effect of conditions without added wheat straw. At 140 DAP, all the cover treatments without added wheat straw (ANM, FM, MBM, and TM) had a lower number of different soil bacterial species than their counterparts with added wheat straw (ANP, FP, MBP, and TP), as seen in Table 31. The higher number of microbial species in cover treatments with added wheat straw could be attributed to the availability of additional organic matter. The fact that these treatments' numbers of species increased from 64 to 140 DAP, strongly supports this observation. As expected, BF and MBM plots hosted the lowest numbers of different bacterial species, whereas TP and MBP illustrated the highest number of microbial species within the soil microbial population (Table 31). The evenness index (Table 31) illustrated a distinct decline in soil microbial abundance from 64 to 140 DAP in methyl bromide plots without added wheat cover (MBM) and bare fallow (BF) plots, while a slight increase in microbial variation could be observed in most of the other cover treatments. It is interesting to note that, 140 DAP, treatments without added wheat straw (ANM, FM, MBM, TM) had a tendency of slightly more variation in microbial populations between species, i.e. more species dominance, thus lower diversity, compared to their counterparts with added wheat straw. Alternatively, taking into account that the increase / decrease of species variation within treatments from 64 to 140 DAP was not statistically significant, it could generally be accepted that cover treatments contained "stable" soil bacterial populations, despite the mentioned bacterial diversity decline in the evenness index in MBM and BF (Table 31). This "stability" in species variation could designate a soil microbial population with the ability to deal with external soil disturbances.

The increase / decrease in soil enzyme activities may be the result of soil physical and chemical changes resulting in a direct expression on microbial biomass and soil enzyme activities. Higher organic matter levels support greater microbial activity because of greater supplies of nutrients and energy (Dick, 1994). No conclusive trend could be drawn from data obtained from only two samplings, but it is interesting to note that there were no statistically significant differences in alkaline phosphatase and urease enzyme activities between treatments with or without added cover between 64 and 140 DAP, except MBM and MBP (Table 32).

Table 32. Average microbial enzyme activities in biocontrol plots 64 and 140 DAP.

Cover Treatment	ß-Glucosidase ^{z}	Alkaline Phosphatase ^{z}	Urease ^{z}
ANM	4.02E+06^{ab}	2.09E+06^{a}	2.27E+01^{a}
ANP	4.02E+06^{ab}	2.06E+06^{a}	2.41E+01^{a}
BF	3.16E+06^{a}	2.12E+06^{a}	1.98E+01^{a}
FM	3.21E+06^{a}	2.31E+06^{a}	2.21E+01^{a}
FP	3.72E+06^{a}	2.15E+06^{a}	2.28E+01^{a}
MBM	3.31E+06^{a}	2.11E+06^{a}	1.46E+01^{a}
MBP	4.69E+06^{b}	2.24E+06^{a}	1.58E+01^{a}
TM	3.64E+06^{a}	1.82E+06^{a}	1.83E+01^{a}
TP	4.11E+06^{ab}	2.05E+06^{a}	2.27E+01^{a}

^{z}Means within a column followed by the same letter do not differ significantly (P = 0.05).

With the exception of a few treatments, cover treatments with added wheat straw cover demonstrated an overall higher enzyme activity, compared to their counterparts without added wheat straw cover (Fig. 14). Treatments with added wheat straw cover (ANP, FP, MBP, TP) showed an increase in ß-glucosidase and alkaline phosphatase activity from 64 to 140 DAP (Fig. 14a) compared to their counterparts without added wheat straw. Although not significant, the higher urease activities (Fig. 14b) in these treatments could be attributed to the increased exudation of nitrogen into the soil due to the degradation of the wheat straw cover. Since ß-glucosidase activity has been found by Bandick & Dick (1999) to be a useful indicator of soil quality, it could be speculated that added wheat straw cover could contribute (over time) to increased soil quality. It is interesting to note that ß-glucosidase and alkaline phosphatase activity in treatments without added wheat straw cover (ANM, FM, MBM, and TM) either decrease, or stabilized between 64 and 140 DAP (Fig. 14).

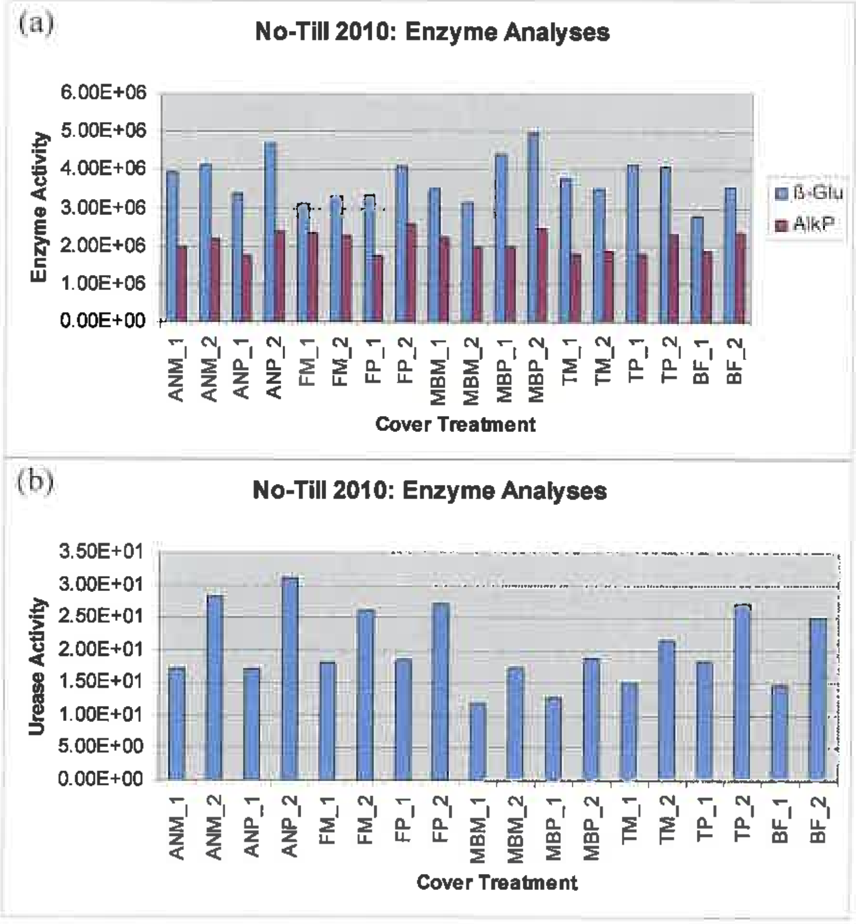

Fig. 14. **ß-glucosidase and alkaline phosphatase (a), and urease (b) activity for wheat straw cover treatments at 64 ("_1") and 140 ("_2") DAP.**

For more reliable answers, any effect of a management system on soil quality and productive capacity is best evaluated using long-term trials (Subbian *et al.*, 2000).

SUMMARY AND CONCLUSIONS

ROTATIONAL EFFECTS

- At 140 DAP, and at harvest, no significant yield benefits were evident. However, canola and crambe resulted in increased yields 28 and 64 DAP. Stooling rye significantly increased yields 64 DAP, but black oat was at no stage superior to the control.

- Maize fallow and bare fallow significantly increased yields 28 DAP, due probably to higher soil temperatures in these treatments during early growth. At 64 DAP, no benefits due to the fallow treatments were evident and 140 DAP and harvest yields in these treatments were markedly lower than in control plots.

- At 64 DAP crown and root rot in maize was significantly reduced by CAN, CR, BO and SR, and 140 DAP root rot in maize following CAN, BO and SR was reduced. Maize fallow (FM) had significantly less root rot 64 DAP than the control (C), but not 140 DAP. Bare fallow (BF) was significantly worse than any other treatment 140 DAP.

- Incidences of *F. graminearum* in roots of maize planted after bare fallow, canola, crambe and fallow without cover were significantly lower than in roots of maize planted after black oat, wheat and stooling rye.

- Rotational treatment had no significant affect on herbivorous nematodes. The total number of herbivores in most of the rotational treatments was significantly more than in the maize after wheat treatment. In both the black oat and canola treatment the population numbers of the bacterivores were significantly higher than that of the fungivores. These two treatments benefited bacterivores over fungivores.

- Differences in root exudate composition from rotational crops caused inconsistency in soil microbial diversity. The lowest number of soil microbial species was present in BF and FM. Enzyme activities were slightly higher in CAN, BO and SR, compared to FM and BF.

BIOCONTROL AGENT EFFECTS

- None of the biocontrol products (ECO, GLIO and OB) resulted in significant yield differences at any stage.

- Crown rot severity was significantly reduced by Eco-T (ECO), Gliogrow (GLIO) and Fungimax plus Organoboost (OB) 64 DAP, but only Gliogrow reduced root rot 64 DAP. At 140 DAP these benefits were not significant anymore. At neither grow stage was ANM+OB different from OB.

- The biocontrol products did not significantly reduce *F. graminearum* in maize crowns and roots. Products also did not significantly affect incidences of *Diplodia/Stenocarpella*. *Phialophora* spp. and *Trichoderma* spp. were more frequently associated with the ECO than the other treatments.

- The biocontrol agents benefited the herbivores 64 DAP.

- ANM+OB and GLIO significantly increased soil microbial species dominance with microbial communities between 64 and 140 DAP. Eco-T indicated a slightly higher potential to increase soil quality and fertility.

CHEMICAL BIOCIDE EFFECTS

- Methyl bromide fumigation significantly increased yields 28 DAP, but this was possibly due to increased soil temperatures during early growth. At 64 DAP no differences relative to the control were evident and 140 DAP and at harvest the effects of methyl bromide minus extra cover were significantly negative. Lower soil moisture content and reduced earthworm populations were probably implicated.

- Anhydrous ammonia significantly increased yields 64 DAP, but not 28 and 140 DAP. At harvest, the benefit of anhydrous ammonia was highly significant.

- Anhydrous ammonia resulted in highly significant increases in plant Mn content.

- Both anhydrous ammonia (ANM) and methyl bromide (MBM) significantly reduced crown and root rot 64 DAP, but not 140 DAP.

- Anhydrous ammonia (ANM) and methyl bromide (MBM) significantly reduced incidences of *F. graminearum* in maize roots. More *Trichoderma* spp. were recorded for the MBM than the ANM and C treatments.

- The lowest number of herbivores was observed in the methyl bromide treatments. The methyl bromide treatment (MBM) was one of two treatments where the incidence of the beneficial nematodes was lower than that of the previous season.

- MBM demonstrated the lowest amount of soil microbial species and the highest level of species dominance within the soil microbial community. MBM treatment also demonstrated the lowest overall enzyme activity.

TILLAGE EFFECTS

- Tillage (rotovation or ripping) significantly increased yields 28 DAP, due probably to increased soil temperatures during early growth. These, effects were not evident at later samplings, however, and in the absence of extra cover, tilled plots were significantly out-yielded by the control.

- Plots ripped without anhydrous ammonia yielded over 2000 kg/ha less than their analogues that received gas.

- Tillage very markedly increased the prevalence of root lodging. Reduced soil moisture content in these treatments, higher temperatures and reduced earthworm populations were probably implicated.

- Tillage (TM) and ripping (CRI) significantly reduced crown and root rot 64 DAP. At 140 DAP the TM treatment had significantly more crown and root rot than CRI and the control (C). At 140 DAP the MBM (tilled + methyl bromide) and ANM (ripped + anhydrous ammonia) had consistently less root rot than just tilled (TM) and ripped (CRI) treatments.

- Soil disturbance significantly reduced incidences of *F. graminearum* in roots, but not crowns. Highest incidences of this fungus and *Phialophora* spp. in crowns were recorded for the TM treatment.

- The highest population numbers of fungivores were found in the tilled minus cover treatment. The tilled minus cover (TM) treatment was one of the few treatments where the beneficial nematodes and not the herbivores, were benefited. Both the incidence and population numbers were significantly higher than that the herbivores.

- Tillage practices with added cover insignificantly increased the number of different bacterial species, whereas ripped and tillage treatments without cover displayed the opposite effect. CRI resulted in the highest alkaline phosphatase activity, whereas MBM and MBP demonstrated the lowest urease activity.

WHEAT STRAW COVER EFFECTS

- The beneficial effects of cover on final grain yield were dramatic and, on average, in treatments that did not receive anhydrous ammonia, exceeded 11%.

- Treatments with extra wheat straw cover consistently had lower soil temperatures, higher soil moisture contents and higher earthworm populations. In addition, they had markedly reduced root lodging.

- Earthworm counts were strongly correlated with infiltration rates and with soil moisture content.

- It is particularly significant that even in maize fallow plots, where the quantity of maize residues approached 9 t/ha, extra wheat straw resulted in significant benefits in terms of soil temperature, soil moisture content, earthworm numbers, infiltration rate and root lodging. The grain yield differential between the FM and FP exceeded 1400 kg/ha.

- Application of extra cover did not show a significant reduction in crown and root rot 64 DAP. At 140 DAP cover significantly reduced crown and root rot for the MBP compared to the MBM and the TP compared to the TM treatment. Differences between ANM and ANP and between FM and FP were not significant.

- Application of wheat straw cover did not significantly affect incidences of *F. graminearum* and *Diplodia/Stenocarpella* in crowns and roots. *Acremonium* spp. increased significantly in crowns when straw cover was applied for the fallow.

- Comparison of these treatments in terms of the number of herbivores, bacterivores, omnivores and predators indicated that the effects 64 DAP were small.

- No meaningful wheat straw trends were observed 64 DAP. Though, chemical biocides with cover (ANP and MBP) had higher numbers of different soil bacterial species, tillage and fallow with cover (TP and FP) had lower numbers of different soil bacterial species than their counterparts. BF and FM displayed a significant reduction in the number of different soil bacterial species between 64 and 140 DAP. However insignificantly small, added wheat straw cover resulted in higher enzyme activities, i.e., slightly higher potential to increase soil quality and fertility.

From an agronomic perspective, the two highlights of this season's findings were the positive effects of anhydrous ammonia on yield and the marked benefits of high biomass cover – cover loads substantially greater than the 30–40% soil cover considered to be adequate for erosion control and targeted by most no-till farmers in this country.

To date, treatments employing anhydrous ammonia as the source of N have on average, over four seasons, out-yielded the control treatment (maize after winter wheat) by over 1200 kg/ha. The mean benefit over standard maize fallows employing 8-10 t/ha of maize residues has been over 2200 kg/ha. This is a very appreciable economic benefit and, as is evident from annual leaf analyses, was not related to differences in N availability; an uninformed opinion that has appeared in the popular agricultural press. Results obtained this season again indicate that the benefits are intimately associated with increased Mn availability. At 28 and 71 DAP, respectively, plant Mn content in anhydrous ammonia treated plots was, on average, 55% and 85% higher than in other treatments and quite clearly this effect was unrelated to the ripping action associated with the application of ammonia gas. The fungicidal properties of Mn are recognised and 64 DAP crown and root rot severity were significantly negatively correlated with plant Mn content 71 DAP. This once again strongly suggests that anhydrous ammonia may have an important role to play in soilborne disease suppression.

Considered particularly significant is the fact that the benefits of anhydrous ammonia are unlikely to be restricted to no-till management. If, as seems probable, increased Mn uptake results from acidification of the rhizosphere as a result of NH_4^+ uptake and also from the conversion of $NH_4 - N$ to $NO_3 - N$ during nitrification, the effect should also be expressed in conventionally tilled management systems. It is also noteworthy that the effects of extra cover have been far less apparent in anhydrous ammonia plots than they have been in other plus- and minus-cover comparisons. Further work is required, however, in order to confirm this observation. There is also a need to assess the effects of anhydrous ammonia application rates and the timing thereof.

Cover effects, the second highlight of this season's results, were again very strongly expressed in methyl bromide treated plots, plots rotovated, but not fumigated and fallow plots. The average yield benefit due to cover was almost 1500 kg/ha, which, if expressed as a percentage, is virtually identical to that noted in the 2008/2009 season and not greatly different to the benefit of a soyabean-wheat-maize rotation relative to a soyabean-fallow-maize treatment, when the benefit of extra cover was first noted in 2007.

From findings obtained this season, it is again evident that the late-season decline in minus-cover plots is directly or indirectly related to reduced earthworm activity in plots not insulated by wheat straw cover, soil surface decreases in moisture content, higher temperatures and possibly, too, to reduced infiltration rates. Doubts persist about the latter possibility, however, as tilled treatments without cover also displayed high infiltration rates. This could be due to errors introduced by lateral water flow in ripped and rotovated plots, but will hopefully be clarified next season. Another important feature of the plus- and minus-cover comparisons is the marked effect that cover and presumably surface moisture content had on root lodging. Lodging is a major practical problem and it is interesting to note that this was a serious problem confronting many no-till farmers in the Bergville/Winterton area this year.

This brings us to some important questions, which necessarily impact on the cover effects we have recorded in three out of four seasons of experimentation. What is the value of residues for other uses and what are the cost implications of generating extra cover? We are not qualified to answer the first question, but would greatly appreciate any guidance readers of this report may be able to give us. Intuitively, is seems most unlikely that the value as grazing for sheep or cattle would exceed the value of 1000 kg/ha of maize grain. Similarly, it seems unlikely that baled wheat straw or maize stover would have a higher value. Burning wheat straw is patently needless and costly unless subsequent maize planting is affected hugely by inadequate equipment.

The cost and climatic limitations to generating extra cover cannot be ignored. In some areas, which experience dry winters and severe frost, the opportunities are not good. However, where a winter cash crop such as wheat is produced, there cannot be a major problem. Also, where irrigation is available, the cost of establishing an alternative winter cover crop might not be unreasonable. Cold tolerant legumes such as vetch, which also provide very significant quantities of N, are prime candidates in this respect, as there would be an additional off-set with respect to fertilizer inputs. Moreover, in a season such as that just experienced, late rains in March-April would probably have ensured good establishment of almost any winter crop planted after soyabean. Such issues deserve consideration and we can only presume that aspects such as this are being researched by other groups. When all is said and done, the biggest limitation in South African agriculture is moisture and cover unquestionably increases water-use efficiency – maximising kilograms of grain produced per litre of water will always be an overriding objective.

Pathogenicity tests conducted for Facet 4 (results in this report) showed that of the fungi frequently isolated from plants collected from the field trial, *F. graminearum*, *P. terrestris*, *Phialophora* spp. and *S. maydis* are the most aggressive pathogens using survival, effect of plant growth, crown rot incidence and root rot severity as indicators of pathogenicity. Of these fungi, *F. graminearum* has been significantly correlated with root rot in the 2006/2007 and 2007/2008 seasons and *S. maydis* was significantly positively correlated with crown rot severity and significantly negatively correlated with grain yield in 2007/2008. Treatments that had the highest crown and root rot severity ratings during this season and the previous season, the bare fallow (BF) and tillage without cover (TM) treatments, had high incidences of either *F. graminearum*, *Phialophora* spp. or *S. maydis* associated with the crowns. Correlating the incidences of these fungi with crown and rot severity and grain yield can be problematic since the plant material used for isolations presents a very small portion of the diseased material. It is also possible that parts of diseased roots are lost during the sampling process and cleaning of the material for transport. Another complication is the difficulty in isolating certain fungi such as *Phialophora* spp. from plant material and incidences recorded with the conventional method of plating can be an underestimation of these fungi in diseased material. The information that we obtained with the pathogenicity study in Facet 4 will allow us to focus more on the aggressive pathogens and perhaps employ molecular technology to obtain more precise data on the quantity of specific pathogens in crowns and roots of maize plants. This technology is unfortunately expensive, but will enable us to elucidate the effect of different management strategies on major soilborne pathogens of maize.

Although not always statistically significant, benefits with regard to increases in grain yield were obtained from including different winter crops in the rotation system. It is, however, important that the winter crop included in a rotation system should be selected with great care. The high incidences of *F. graminearum*, which is a pathogen of grass crops such as maize, wheat, black oat and stooling rye causing diseases such as scab and cob and stalk rot, on diseased maize crowns and roots in our field trial should be a concern when choosing a winter crop to rotate with maize. This fungus poses serious risks due to the production of mycotoxins in infected grain and the effect this may have on human and animal health. Molecular characterisation of isolates of *F. graminearum* obtained from maize crowns and roots will be available for our next report and will indicate whether these isolates belong to the same or different lineages of the *F. graminearum* isolates involved in wheat scab and cob and stalk rot of maize.

Including winter crops can reduce crown and root rot severity, but these benefits are not always realised statistically in terms of grain yield. Since soilborne plant pathogens survive in stubble and organic material in soil it seems that the short break away from maize using winter crops is possibly not enough to significantly reduce pathogens responsible for crown and root rot of maize. However, including winter crops that are not hosts of soilborne pathogens of maize will change the microbial composition of soil which may enhance the antagonistic potential of the soil against soilborne pathogens of maize, because it is well known that more diverse rotation systems are less prone to soilborne disease problems.

Earthworms have been implicated in the reduction of soilborne diseases in other crops. In our trial, earthworm counts were significantly negatively correlated with lodging. The fact that earthworm counts were also significantly negatively correlated with crown and root rot severity and soil temperature and significantly positively correlated with soil moisture showed that strategies to increase soil moisture and reduce soil temperature will enhance earthworm counts in the field and thereby reduce crown and root rot of maize. However, the mechanisms involved in the reduction of these diseases in maize with an increase in earthworm counts needs to be elucidated. Clearly, the effects of cover on earthworm counts are a major confounding issue.

An important finding, confirming the previous seasons' results is the increase in crown and root rot severity as a result of moisture stress in treatments without cover. This can be a result of decreased populations of beneficial organisms in soil or that drought-stressed plants are rendered more susceptible to attack by certain soilborne pathogens. Incidences of *F. graminearum* were positively correlated with soil moisture and incidences of *Phialophora* spp. negatively correlated, which indicates that soil moisture affects pathogens differently. This aspect needs to be investigated further to assist us in understanding the factors predisposing maize plants to crown and root rot and to be able to develop an effective management strategy against these diseases.

The monitoring and observation of the effect the different treatments had on the nematode fauna helped for a better understanding of the way in which nutrients and therefore, energy, are recycled and transferred within the soil ecosystem, as well as the impacts that agricultural management has on the soil foodweb. Many of the functions provided by nematodes in the ecosystem are a direct result of their feeding activity and metabolic processes and, therefore, soil nematodes play an important role in the soil food web. Being present as plant parasites, consumers of fungi and bacteria and as predators, they are excellent bioindicators of the structure of the soil food web and were used as such in the present study. In this study an increase, over several seasons, in the level of diversity of soil organisms can already be seen as increased population numbers and incidence of beneficial nematodes (bacterivores and fungivores) in almost all treatments. It is, however, unclear how long it will take for these practices to change the health of the soil and what impact they will have in the long term. The very low numbers of the predacious and omnivorous nematodes is still worrying. The same trend was observed in other studies where conversion to reduced tillage did not show immediately probably because predators may be slow colonizers, have longer life cycles and balance in the soil web may require considerable time.

As it is known that no-tillage favours foodwebs dominated by fungi and fungus-feeding fauna, the higher incidence of bacterivores in especially the rotational treatments, poses some questions. For the bacterivores to benefit over the fungivores there has to be some source of bacteria in the soil and roots.

Different levels of soil disturbances have different effects on soil microbial populations. If our goal is to preserve biodiversity in agricultural soils, we need to understand how diversity is impacted by different management strategies. Based on results obtained from samples collected during 2009/2010, it is difficult to determine which practice is most beneficial or detrimental to soil microbial populations, due to the presence of a large number of variables. The sensitivity of soil microbial diversity and enzymatic activity could be clearly observed in the influence that the various treatments had on these indicators of soil health and soil fertility over a period of time. Based on the results obtained during samplings taken 64 and 140 DAP, differences in root exudate composition were responsible for inconsistencies in soil microbial diversity, but enzyme activites were slightly more elevated in rotational crops, compared to FM and BF. Chemical biocides resulted in overall lower microbial diversity and enzymatic activity, compared to the control treatment, while no significant differences were visible in the biocontrol treatments. The disturbance of soil and the absence or low percentage of winter crops and associated lower levels of organic matter content of the topsoil covers, had an insignificant impact on soil biochemical properties (enzymatic activity) and soil microbial diversity. The positive effect of added straw cover and crop rotation is evident, especially on enzyme activities relating to soil quality. This may result in increased mineralisation rates and hence increased nutrient recycling. In the long term, these factors could result in increased soil quality and fertility, which could have a significant effect on sustainable agriculture.

The importance of monitoring soil microbial diversity and enzymatic activity over time can not be over-emphasized. It is recommended that these trends be monitored over time in order to attain a more complete reflection of the impact that the different treatment groupings would have on microbial diversity and enzymatic activity as indicators of soil fertility and health. Enzyme activities obtained as trends over time in the appendix, clearly indicate the increased activity of ß-glucosidase and urease enzyme activities. Unfortunately, no clear-cut trends could be observed in either soil microbial diversity or phosphatase enzyme activities. A better understanding of the role and impact of agricultural management practices on soil microbes could lead to increased soil quality and fertility, resulting in significant effects on the sustainability of particular cropping systems.

Finally, in order to better understand the critical processes involved in no-till management and the effects of cover, further changes and additions will be made to the experimental protocol during the 2010/2011 season. As has already been explained, the method of determining infiltration rate will be altered in order to reduce the possible influence of lateral water movement in plots that have been rotovated or ripped. In addition, two further measurements will be included. One, using 1-m access tubes, will enable us to measure subsoil moisture content at any stage during the season and the other, employing 0-50-mm soil samples, will provide critical information on the carbon and nutritional content of the soil immediately below the mulch layer. It is likely that it is in this zone that the most meaningful changes are occurring.

FACET 4

THE RELATIVE IMPORTANCE OF FUNGI FREQUENTLY ASSOCIATED WITH DISEASED MAIZE CROWNS AND ROOTS AS SOILBORNE PATHOGENS OF MAIZE AND ROTATION CROPS, AND THE INTERACTION BETWEEN FUNGAL PATHOGENS AND PARASITIC NEMATODES ON MAIZE

EXECUTIVE SUMMARY

Pathogenicity tests were conducted with 420 isolates (single-spore or hyphal-tipped) selected from 1 882 isolates representing all fungi (more than 75 fungal species) associated with diseased maize crowns and roots in the field trials conducted in Winterton, KwaZulu-Natal. Trials were conducted in a glasshouse at 18° C night and 28° C day temperatures using a pasteurised soil, river sand and perlite medium and a 0.5% sand-bran inoculum of each fungus. The maize cultivar, PHI 32D96B, was used in the trials and survival, growth, crown and root rot severity were recorded 3 weeks after planting (inoculation). A large number of fungi isolated from diseased maize crowns and roots caused crown and root rot and reduced survival and growth of maize plants. Fungi that significantly reduced survival of seedlings compared with the control were *Fusarium avenaceum* (78% survival), *F. graminearum* (68%), *F. pseudograminearum* (87%), *Pythium mamillatum* (87%), *Trichoderma. asperellum* (86%), *T. dorotheae* (87%), *Pythium spinosum* (85%), *P. torulosum* (77%), *P. ultimum* (77%), *Rhizoctonia solani* AG-2-2 (83%), and Unidentified 6 (87%). The highest mean root rot ratings were recorded for *F. graminearum* (2.47), *F. avenaceum* (1.34), *Phialophora* spp. (1.33), *Pyrenochaeta terrestris* (1.27), *R. solani* AG-2-2 (2.19), *Stenocarpella maydis* (2.24) and Unidentified 1 (1.57), Unidentified 3 (1.33), Unidentified 4 (1.06) and Unidentified 7 (1.88). Fungi that caused significant crown rot compared to the control were *F. graminearum* (68%), *S. maydis* (56%), *R. solani* AG-2-2 (52%), *F. avenaceum* (48%), Unidentified 7 (36%), Unidentified 5 (33%), Unidentified 4 (18%), *Phialophora* spp. (16%), *P. terrestris* (10%), *Bipolaris* spp. (7%), *Macrophomina phaseolina* (6%), Unidentified3 (6%), *F. subglutinans* (6%) and *F. proliferatum* (6%). Fungi that significantly reduced growth were *Apergillus* sp., *F. avenaceum, F. graminearum, Phialophora* spp. *P. aristosporum, P. arrhenomanes,*

P. periilum, *P. rostratifinges*, *P. torulosum*, *R. solani* AG-2-2, *S. maydis*, Unidentified 1, Unidentified 3, Unidentified 5, Unidentified 7. All the *Trichoderma* spp. except for *T. asperellum* significantly improved growth compared to the control. 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. Isolates within the different fungal groups often differed significantly with regard to their ability to reduce survival and growth and to cause crown and root rot. 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 other researchers locally and 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.

INTRODUCTION

A number of fungi have been isolated from diseased crowns and roots of maize in our field trials in KZN for the past few seasons (Lamprecht *et al.*, 2006, 2007, 2008, 2009), and by other researchers in other parts of South Africa (Du Toit, 1968; Kruger, 1970; Scott, 1982; Deacon & Scott, 1983; Chambers, 1987a,b; Smit, 1998; Smit, Van Rensburg & Rijkenberg, 1997), but information on the importance of these fungi as soilborne pathogens under local conditions is limited. Chambers (1987b) evaluated the fungi that he isolated from maize roots for their ability to reduce seedling survival, but not their ability to cause root rot. According to White (1999) root rot of maize is a complex disease, but there are four diseases that are distinct viz. Pythium root rot, Rhizoctonia crown and brace root rot, Fusarium root rot, and red root rot (caused by *Pyrenochaeta terrestris*). In our studies thus far we have obtained a range of fungi, with the fungi most frequently isolated from diseased crowns and roots being *Acremonium* spp., *Fusarium equiseti*, *F. graminearum*, *F. oxysporum*, *F. proliferatum*, *F. solani*, *F. subglutinans*, *Phialophora* spp., *Pyrenochaeta terrestris*, *Stenocarpella maydis* and *Trichoderma* spp. Fungi that are less frequently isolated, but that have been recorded as important pathogens of maize, are *Pythium* spp. and *Rhizoctonia* spp. (White, 1999). It is, however, interesting to note that *F. graminearum* is generally regarded as a foliage pathogen (cob and stalk rot of maize and scab of wheat) and not a soilborne pathogen of maize (White, 1999). References to *F. graminearum* as a soilborne pathogen of maize are often contradictory with certain researchers regarding the fungus as an important root rot pathogen (Miller, 1964) and others not (Hornby & Ullstrup, 1967). We have isolated this fungus frequently from roots with incidences as high as 41% of root pieces plated yielding the fungus by the end of the 2006 growing season (Lamprecht *et al.,* 2006). Similarly, *Stenocarpella maydis* is a stalk and cob rot pathogen (White, 1999), but we quite frequently isolate this fungus from crowns and roots of maize later in the growing season (Lamprecht *et al*., 2008, 2009, see Facet 1). It is, therefore, important to determine the importance of this fungus as a soilborne pathogen of maize and to compare its virulence with those of the other fungi frequently associated with diseased maize crowns and roots. Of the other fungi frequently isolated, *F. equiseti, F. oxysporum* and *F. solani* are regarded as weak pathogens of maize roots, and *F. oxysporum* has also been listed as a wound pathogen of maize (Palmer & Kommedahl, 1969; Warren & Kommedahl, 1973). *Fusarium proliferatum* and *F. subglutinans* are listed as cob rot pathogens of maize and the importance of these fungi as root rot pathogens of maize is not clear. *Pyrenochaeta terrestris* is regarded as a primary pathogen in the complex causing red root rot of maize, but its relative importance compared to other fungi associated with diseased maize crowns and roots needs to be elucidated. We have isolated eleven *Pythium* spp. in our previous studies viz. *P. acanthicum, P. aristosporum, P. arrhenomanes, Pythium* HS Group, *P. irregulare, P. mamillatum, P. periilum* and *P.rostratifinges, P. spinosum, P. torulosum and P. utimum*. All of these except *P. periilum* have been recorded as soilborne pathogens of maize in other countries (Zhang, Chen & Yang, 1998; Van Zeeland, Lamers & van Dijk, 1999; White, 1999). However, the importance of these *Pythium* spp. on maize in South Africa is not known. *Rhizoctonia* spp. and anastomosis groups that we isolated include, *Rhizoctonia solani* AG-2-2, *Rhizoctonia* AG-A, *Rhizoctonia* AG-F, *Rhizoctonia* AG-R and *R. zeae*. According to researchers in other countries, *Rhizoctonia solani* AG-2-2IIIB is the most important *Rhizoctonia* pathogen of maize (Sumner & Bell, 1982).

The most dominant fungi isolated by us in our previous studies, especially during the early growth stage were *Trichoderma* spp. *Trichoderma* spp. have been listed as both pathogens of maize and biocontrol agents against maize diseases (McFadden & Sutton, 1975; Elad, Zvieli & Chet, 1986; Mao *et al.,* 1997). In our studies we obtained nine species of *Trichoderma* viz. *T.asperellum, T. dorotheae, T. hamatum, T. harzianum, T. koningiopsis, T. ovalisporum, T. spirale, T. theobromicola* and *T. virens*. McFadden & Sutton (1975) showed that *T. koningii, T. harzianum* and *T. hamatum* can produce first internode lesons in maize seedlings. The information on *T. koningii, T. harzianum* and *T. hamatum* as pathogens of maize is limited to Ontario, Canada, and it is uncertain to what extent *Trichoderma* spp. can cause disease problems in other maize producing countries.

In order to develop a sustainable management strategy against soilborne diseases of maize we need to determine the relative importance of the fungi most frequently associated with diseased crowns and roots of maize and rotation crops. The objective for this season was to conduct glasshouse trials to determine the importance as soilborne pathogens of the fungi obtained from maize.

MATERIALS AND METHODS

Of the 1 882 fungal isolates selected from isolations, 800 were tested in preliminary trials under laboratory conditions. Of these isolates, 420 were selected and single-spored or hyphal-tipped for formal pathogenicity tests under glasshouse conditions, with the emphasis on fungi that have been frequently associated with diseased maize crowns and roots in the field trials at Bergville and Winterton, as well as fungi previously recorded to be pathogenic on maize crowns and roots by other researchers.

Soil (7000 kg) was transported from the experimental site near Winterton to our laboratories in Stellenbosch. A pasteurised potting mix consisting of soil, river sand and coarse perlite (1:1:1) was used in the pathogenicity tests. Trials were conducted under glasshouse conditions at 28° C day and 18° C night temperatures (Fig. 1).

An inoculation technique developed by Lamprecht (1986) using sand-bran as a medium to grow the respective fungi for inoculum production was used. The growth medium was inoculated with the sand-bran at a 0.5% w/w (inoculum/growth medium) one day before planting. The maize cultivar PHI 32D96B was used in the trials and planted at 10 seeds per pot. Pots were watered on alternative days and plants evaluated for crown and root rot 3 weeks after inoculation. Root rot severity was rated as follows: 0 = roots healthy, 1 = >0 – 25% root rot, 2 = >25 – 50% root rot, 3 = >50 – 75% root rot, 4 = >75 – 100% root rot. The number of plants that developed crown rot and the survival and growth were also determined 3 weeks after planting.

The trials were randomised block designs and data were analysed using ANOVA and the Student-t least significant difference at 5% significance level was calculated to compare means.

Fig. 1. Pathogenicity trial in glasshouse at the Vredenburg Research Centre of the ARC-PPRI in Stellenbosch.

RESULTS

Results on the effect of the different fungi on survival of seedlings, root rot severity, crown rot incidence and growth reduction are given in Table 1.

Table 1. Effect of fungi on survival, root rot severity, incidence of crown rot and growth reduction of maize under glasshouse conditions.

Fungus	No. of isolates tested	Survival (%)^{w}	Root rot severity^{x}	Crown rot Incidence (%)^{y}	Growth reduction^{z}
Control		96.7a-d	0.00q	0.00h	
Acremonium spp.	10	97.0a-d	0.00q	0.00h	NS
Alternaria sp.	1	100.0a	0.00q	0.00h	NS
Aspergillus sp.	1	100.0a	0.00q	0.00h	SR
Bipolaris spp.	6	93.3a-g	0.28no	6.93fg	NS
Chaetomium spp.	2	95.0a-f	0.00q	0.00h	NS
Cladosporium spp	4	96.7a-d	0.00q	0.00h	NS
Colletotrichum sp.	3	95.6a-f	0.00q	0.00h	NS
Coprinus spp.	4	99.2ab	0.00q	0.00h	NS
Curvularia spp.	1	93.3a-g	0.00q	0.00h	NS
F. avenaceum	2	78.3jk	1.34e	48.15c	SR
F. cerealis	2	98.3ab	0.54lm	0.00h	NS
F. compactum	1	90.0b-h	0.00q	0.00h	NS
F. dimerum	3	92.2a-h	0.00q	0.00h	NS

Fungus	**No. of isolates tested**	**Survival (%)^{w}**	**Root rot severity^{x}**	**Crown rot Incidence (%)^{y}**	**Growth reduction^{z}**
F. dlamini	1	100.0a	0.00q	0.00h	NS
F. equiseti	6	97.8a-c	0.00q	0.00h	NS
F. globosum	1	96.7a-d	0.00q	0.00h	NS
F. graminearum	34	73.3k	2.47a	68.25a	**SR**
F. nygamai	10	91.2a-h	0.00q	0.00h	NS
F. oxysporum	31	98.5ab	0.27no	0.00h	NS
F. poae	1	93.3a-g	0.00q	0.00h	NS
F. proliferatum	10	94.8a-f	0.17op	5.94fg	NS
F. pseudograminearum	1	86.7e-i	0.58kl	0.00h	NS
F. scirpi	3	96.7a-d	0.00q	0.00h	NS
F. semitectum	3	98.9ab	0.00q	0.00h	NS
F. solani	10	96.0a-e	0.08pq	0.00h	NS
F. subglutinans	40	97.3a-c	0.98fg	5.97fg	NS
F. verticillioides	10	92.0a-h	0.02pq	2.26gh	NS
***Fusarium* sp. 1**	10	93.0a-g	0.04q	0.33h	NS
***Fusarium* sp. 2**	3	98.9ab	0.00q	0.00h	NS
Gliocladium roseum	5	97.3a-c	0.00q	0.00h	NS
Macrophomina phaseolina	4	95.0a-f	0.25no	6.30fg	NS
***Mucor* spp.**	4	94.2a-g	0.00q	0.00h	NS
***Neocosmospora* sp.**	1	100.0a	0.00q	0.00h	NS
***Nigrospora* sp.**	1	100.0a	0.00q	0.00h	NS
***Penicillium* sp.**	1	100.0a	0.00q	0.00h	NS
***Phialophora* spp.**	17	97.3a-c	1.33e	15.96e	**SR**
***Phoma* spp.**	15	90.0b-h	0.07pq	2.30gh	NS
***Pithomyces* sp.**	1	100.0a	0.00q	0.00h	NS
Pyrenochaeta terrestris	17	98.4ab	1.27e	9.56f	NS
Pythium acanthicum	2	98.3ab	0.00a	0.00h	NS
P. aristosporum	6	93.9a-g	0.91f-h	2.49gh	**SR**
P. arrhenomanes	4	90.0b-h	0.89f-h	4.94f-h	**SR**
***Pythium* HS Group**	1	96.7a-d	0.66j-l	0.00h	NS
P. periilum	7	89.5b-i	0.66j-l	0.00h	**SR**
P. irregulare	15	87.3d-i	0.72i-k	0.00h	NS
P. mamillatum	5	86.7e-i	0.78h-j	0.00h	NS
P. rostratifinges	2	93.3a-g	1.0fg	15.16e	**SR**
P. spinosum	4	85.0g-j	0.87g-i	0.00h	NS
P. torulosum	2	76.7jk	0.91f-h	0.00h	**SR**
P. ultimum	1	76.7jk	0.91f-h	0.00h	NS
***Rhizoctonia* AG-A**	5	96.7a-d	0.62j-l	0.00h	NS
***Rhizoctonia* AG-F**	4	96.7a-d	0.39mn	0.00h	NS
***Rhizoctonia* AG-R**	4	91.2a-h	0.90f-h	0.00h	NS
***R. solani* AG-2-2**	12	82.5h-k	2.19b	52.47bcd	**SR**
R. zeae	1	96.7a-d	0.76h-j	3.70gh	NS
Stenocarpella maydis	11	91.2a-h	2.24b	55.76b	**SR**
Sterile	2	96.7a-d	0.00q	0.00h	NS
Trichoderma asperellum	5	86.0f-j	0.00q	0.00h	NS
T. dorotheae	1	86.7e-i	0.00q	0.00h	SI
T. hamatum	4	91.7a-h	0.00q	0.00h	SI
T. harzianum	4	95.8a-e	0.00q	0.00h	SI
T. koningiopsis	5	92.0a-h	0.00q	0.00h	SI
T. ovalisporum	4	88.3c-h	0.00q	0.00h	SI

Fungus	**No. of isolates tested**	**Survival (%)**[w]	**Root rot severity**[x]	**Crown rot Incidence (%)**[y]	**Growth reduction**[z]
T. spirale	2	98.3ab	0.00q	0.00h	SI
T. theobromicola	2	88.3c-h	0.00q	0.00h	SI
T. virens	2	93.3a-g	0.00q	0.00h	SI
***Ulocladium* sp.**	1	90.0b-h	0.00q	0.00h	NS
Unidentified 1	1	93.3a-g	1.57d	0.00h	**SR**
Unidentified 2	1	96.7a-d	0.00q	0.00h	NS
Unidentified 3	2	91.7a-h	1.33e	6.02fg	**SR**
Unidentified 4	3	96.7a-d	1.06f	18.18e	**SR**
Unidentified 5	1	100.0a	0.87g-i	33.33d	NS
Unidentidied 6	1	86.7e-i	0.00q	0.00h	NS
Unidentified 7	11	98.2a-c	1.88c	35.56d	**SR**
Unidentified 8	2	93.3a-g	0.00q	0.00h	NS
LSD (P = 0.05)		9.82	0.165	5.189	NS

[w] Survival out of ten seedlings/pot and three pots /treatment were used.

[x] Rating scale where 0 = Healthy roots, 1 = >0 – 25% root rot, 2 = >25 – 50% root rot, 3 = >50 – 75% root rot, 4 = >75 – 100% root rot.

[y] Number of plants with crown rot/number of plants surviving x 100.

[z] SR = Significant reduction compared to the control at P = 0.05, SI = Significant increase compared to the control; NS = Not significant reduction compared to the control at P = 0.05.

Fungi that significantly reduced survival of seedlings were (within brackets the lowest to the highest survival rates recorded), *F. avenaceum* (73–83%), *F. graminearum* (27-100%), *F. pseudograminearum* (87%), *P. mamillatum* (80–90%), *T. asperellum* (77–93%), *T. dorotheae* (87%), *P. spinosum* (70–97%), *P. torulosum* (63–90%), *P. ultimum* (76%), *R. solani* AG-2-2 (60–100%), and Unidentified 6 (87%). Survival rates were the lowest for *F. graminearum* (73 %), *F. avenaceum* 76%), *P. torulosum* (77%), *P. ultimum* (77%) and *R. solani* AG-2-2 (83%) and survival rates of these fungi differed significantly from that of *F. pseudograminearum, P. mamillatum, T. dorotheae*, and Unidentified 6. Isolates within *F. graminearum, P. spinosum*, and *R. solani* AG-2-2 differed significantly with regard to the reduction in survival that they caused (data not shown).

Mean root rot ratings for the different species varied from 0 to 2.47. The highest root rot ratings (within brackets the lowest and highest survival rates recorded) were recorded for *F. avenaceum* (0.04–2.81), *F. graminearum* (0.43–3.56), *Phialophora* spp. (1.03–1.97), *P. terrestris* (0.79–2.10), *R. solani* AG-2-2 (1.03–3.84), *S. maydis* (1.93-2.56) and Unidentified 1 (1.57), Unidentified 3 (1.10–1.57), Unidentified 4 (0.03-1.66) and Unidentified 7 (1.10–2.55). These fungi all had mean root rot ratings of more than 1.0. Root rot ratings of *F. graminearum* were the highest and differed significantly from those recorded for the other fungi. Root rot severities recorded for *R. solani* AG-2-2 and *S. maydis* were significantly higher than those recorded for the other fungi mentioned above, except *F. graminearum.* Ratings for Unidentified 1 and

Unidentified 7 were also significantly different from each other and significantly higher than those recorded for *F. avenaceum*, *Phialophora* spp, *P. terrestris*, and Unidentified 3 and Unidentified 4. Ratings recorded for *Phialophora* spp. did not differ significantly from those recorded for *P. terrestris* (Table 1). Isolates within all these fungal species differed significantly with regard to the root rot that they caused, except for isolates within Unidentified 1 and Unidentified 3 (data not shown).

Fungi that caused significant crown rot compared to the control were *F. graminearum* (68.25%), *S. maydis* (55.76%), *R. solani* AG-2-2 (52.47%), *F. avenaceum* (48.15%), Unidentified 7 (35.56%), Unidentified 5 (33.33%), Unidentified 4 (18.18%), *Phialophora* spp. (15.96%), *P. terrestris* (9.58%), *Bipolaris* spp (6.93%), *Macrophomina phaseolina* (6.30%), Unidentified3 (6.02%), *F. subglutinans* (5.97%) and *F. proliferatum* (5.94%). *F. graminearum* caused the highest percentage crown rot, and differed significantly from the other fungi, whereas *R. solani* AG-2-2 and *S. maydis* did not differ with regard to the percentage crown rot, but *Phialophora* spp. caused significantly more crown rot than *P. terrestris*, *F. proliferatum* and *F. subglutinans*. The lowest and highest percentages of crown rot recorded for the fungi were *Bipolaris* spp. (0–17%), *F. avenaceum* (0-96%), *F. graminearum* (0-100%), *F. proliferatum* (0–26%), *F. subglutinans* (0-34%), *M. phaseolina* (3-10%), *Phialophora* spp. (0–39%), *P. terrestris* (0-22%), *Rhizoctonia* AG-2-2 (3-100%), *S. maydis* (24-86%), Unidentified 3 (0-12%), Unidentified 4 (14-20%), Unidentified 5 (33%) and Unidentified 7 (0-96%). Isolates within each of these fungi differed significantly with regard to crown rot incidence, except for isolates within *Biploaris* spp., *F. proliferatum*, *M.phaseolina* and Unidentified 4 (data not shown).

Fungi that significantly reduced growth compared to the control, were *Apergillus* sp., *F. avenaceum, F. graminearum, Phialophora* spp. *P. aristosporum, P. arrhenomanes, P. periilum, P. rostratifinges, P. torulosum, R. solani* AG-2-2, *S. maydis,* Unidentified 1, Unidentified 3, Unidentified 5, Unidentified 7. All the *Trichoderma* spp. except for *T. asperellum* significantly improved growth.

The pathogenicity results of fungi frequently isolated from maize in Facet 1 as well as fungi previously indicated to be pathogenic on maize will be discussed in detail below.

Although *Acremonium* spp. are often frequently isolated from crowns and roots of maize in our trial (see Facet 1), these fungi did not reduce survival or growth and no crown and root rot were recorded (Table 1). In results obtained previously, incidences of these fungi seem to decrease from the beginning to the end of the season. *Acremonium* spp. were isolated by Chambers (1987a, b) from maize roots and he demonstrated that these fungi did not cause significant reduction in seedling emergence. The specific species of *Acremonium* associated with maize in our trial is currently being determined. In studies by other researchers it seems that *Acremonium strictum* can be a pathogen of maize and that *A. zeae* is endophytic in maize and can protect maize from infection by pathogenic fungi (Tagne, *et al.*, 2002; Wicklow & Poling, 2009). This season, significantly more *Acremonium* spp. were isolated from tillage, fallow and methyl bromide plots with extra straw cover (TP, FP, MBP) compared to similar treatments without extra cover. Since this was not the case the previous season it should be confirmed to be of any value.

The most aggressive *Fusarium* spp. were *F. graminearum* and *F. avenaceum*. Both species caused a significant reduction in survival and plant growth, and significant root and crown rot (Fig. 2a, b). *Fusarium graminearum* caused significantly more crown and root rot than the other virulent fungi in the trial (Table 1). *Fusarium graminearum* has been very frequently isolated from diseased maize crowns and roots for the duration of the field study. The fungus has been recorded to cause seedling blight and root rot of maize (Du Toit, Kirby & Pedersen, 1997; Munkvold & O'Mara, 202; Moreno-Gonzalez *et al.*, 2004). Xu & Zhang (1985), Asran & Buchenhauer (2003) and Moreno-Gonzalez *et al.* (2004) consider *F. graminearum* to be an important root rot pathogen of maize. Similarly, Miller (1964) considers *F. graminearum* to be of prime importance as a soilborne pathogen of maize, but Hornby & Ullstrup (1967) only occasionally isolated *F. graminearum* from maize roots. In a study on lodging of maize plants, Andres Ares *et al.* (2004) isolated *F. semitectum*, *F. graminearum*, *F. culmorum*, *F. solani*, and *F. verticillioides* from plants showing lodging. In pathogenicity tests they showed that *F. graminearum* was the most pathogenic fungus considering either root rot or seedling growth reduction. It appears that this fungus is favoured by hot, humid conditions, since Lamprecht (2007) did not isolate *F. graminearum* frequently from diseased crowns and roots of maize in Vaalharts, but frequently isolated it from diseased crowns and roots of maize in KwaZulu-Natal (Lamprecht *et al.*, 2006, 2007, 2008, 2009). During the 2007/2008 season, the incidence of the fungus at the third sampling time was significantly negatively correlated with root rot severity. Similarly, during the 2006/2007 season, incidence of this fungus was significantly correlated with crown and root rot severity, which strongly suggests that it plays an important role in crown and root rot of maize. The fungus was previously isolated from maize roots in South Africa by Chambers (1987a, b). He found that the fungus did not cause significant reduction in seedling emergence, but he did not evaluate the capacity of the fungus to cause root rot. In our study certain *F. graminearum* isolates were also weak pathogens (data not shown).