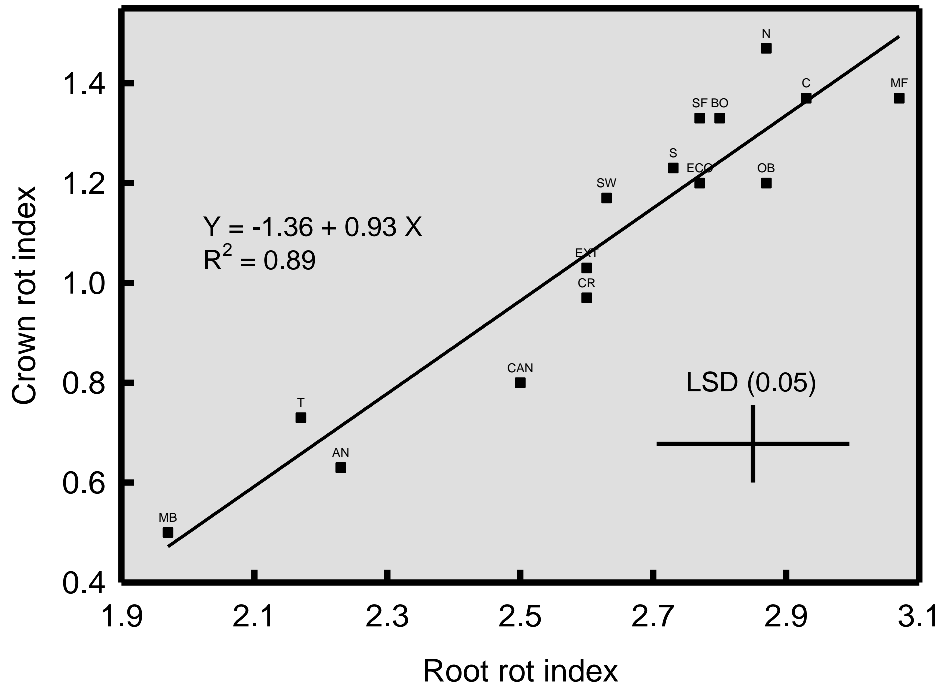


Fig. 13a. **Linear relationship between crown and root rot severities 70 days after planting.**

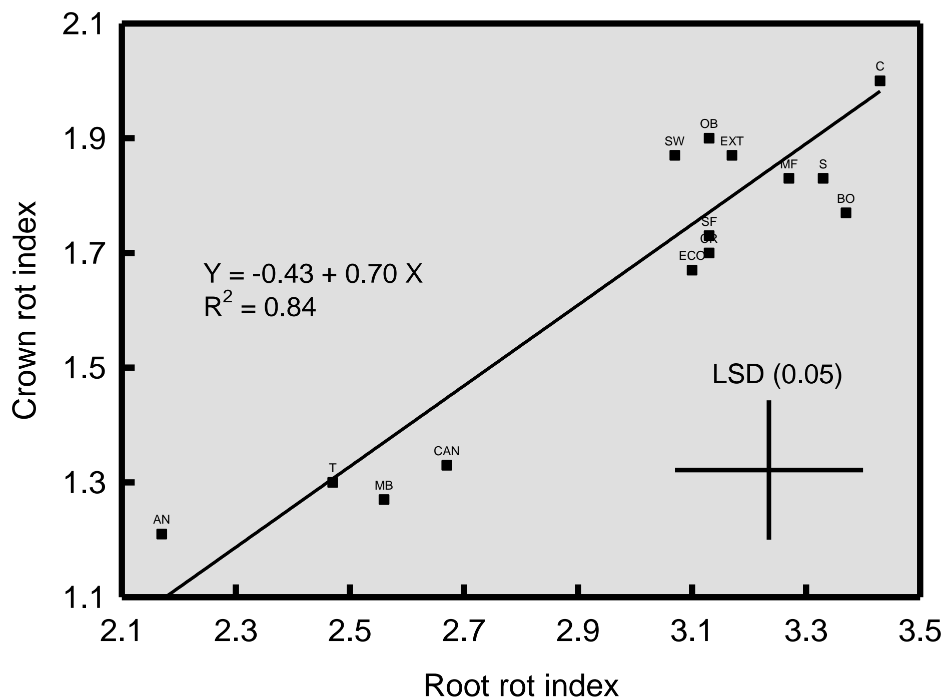


Fig. 13b. **Linear relationship between crown and root rot severities 100 days after planting.**

Incidence of fungi in crowns and roots

A number of fungi were isolated from crowns and roots in this study. Fungi infrequently isolated are listed in Table 8 and were not subjected to statistical analyses. Although *Acremonium* spp. and *Pythium* spp. were also infrequently obtained we included these fungi in the statistical analyses in order to compare their occurrence with results obtained during the previous season (Lamprecht *et al*., 2006). Fungi most frequently isolated from crowns and roots were *Fusarium equiseti*, *F. graminearum*, *F. nygamai*, *F. oxysporum*, *F. proliferatum, F. solani*, *F. subglutinans*, *Pyrenocheata terrestris*, and *Trichoderma* spp. Treatments and sampling time affected the incidences of some of these fungi in the crowns and roots (Table 9). All fungi were isolated from crowns and roots and *Acremonium* spp., *F. proliferatum* and *F. subglutinans* were isolated more frequently from crowns than roots of plants. The fungi isolated from crowns and roots during this season were similar to those obtained during the previous season (Lamprecht *et al*., 2006). All of the fungi frequently isolated, except *F. nygamai*, have been previously recorded as soilborne pathogens of maize. Of the less frequently isolated fungi, *Acremonium* spp. *F. verticillioides*, *Pythium* spp. and *Rhizoctonia* spp. are known as soilborne pathogens of maize (Sumner & Bell, 1982; Ramsey, 1990). Soilborne diseases of maize include seed rot, seedling blight, damping-off, crown and root rot (White, 1999). According to White (1999) root rot of maize is a disease complex, but there are four root diseases that are distinct viz., Pythium root rot, Rhizoctonia crown and brace root rot, Fusarium root rot, and red root rot.

Table 8. Fungi infrequently isolated from crowns and roots of maize plants at Winterton.

Fungus	Crowns[z]	Roots[z]
Acremonium spp.	+	+
Alternaria spp.	+	+
Aspergillus spp.	+	+
Bipolaris spp.	-	+
Chaetomium spp.	+	+
Cladosporium cladosporioides	+	+
Diplodia spp.	+	+
Epicoccum spp.	+	+
Fusarium scirpi	-	+
Fusarium spp. (unidentified)	+	+
Fusarium verticillioides	+	+
(syn. *Fusarium moniliforme*)		
Gliocladium roseum	+	+
Macrophomina phaseolina	+	+
Mortierella spp.	+	+
Neocosmospora vasinfecta	+	+
Penicillium spp.	+	+
Phoma spp.	+	+
Pythium spp.	+	+
Rhizoctonia spp.	+	+
Rhizopus spp.	+	+
Sterile fungi	+	+
Unidentified fungus	+	+

[z]+ = Fungus isolated; - Fungus not isolated

Table 9. Significant levels (P values) of the effect of treatment and sampling time on the incidences of fungi in crowns and roots.

Factors	Plant Part	Fungi										
		Acrem	Fequi	Fgram	Fnyga	Foxys	Fprol	Fsola	Fsubg	Pyren	Pythi	Trich
Treatment (T)	Crown	NS	NS	0.0172	NS	NS	NS	NS	NS	NS	NS	NS
	Root	NS	0.0086	0.0009	NS	NS	0.0340	NS	NS	NS	NS	NS
Sampling Time (S)	Crown	NS	NS	0.0118	NS	0.0041	NS	NS	NS	NS	NS	0.0053
	Root	NS	<0.0001	<0.0001	NS	<0.0001	NS	<0.0001	0.0273	0.0022	0.0179	<0.0001
T x S	Crown	NS	NS	NS	NS	0.0010	NS	NS	0.0253	NS	NS	NS
	Root	NS	<0.0001	NS	NS	NS	0.0006	NS	NS	NS	NS	NS

[y] Acrem = *Acremonium* spp., Fequi = *Fusarium equiseti*, Fgram = *Fusarium graminearum*, Fnyga = *Fusarium nygamai*, Foxys = *Fusarium oxysporum*, Fprol = *Fusarium proliferatum*, Fsola = *Fusarium solani*, Fsubgl = *Fusarium subglutinans*, Pyren = *Pyrenochaeta terrestris*, Pythi = *Pythium* spp., Trich = *Trichoderma* spp.

[z] NS = not significant

Trichoderma spp., followed by *F. oxysporum*, *F. graminearum* and *P. terrestris* were the fungi most frequently isolated from crowns and roots of plants in this trial. All these fungi were more frequently isolated from the roots than from the crowns of plants (Table 10, 11 & 12). Averaged over sampling times, the different treatments did not significantly affect the incidences of *Trichoderma* spp. in crowns and roots, but incidences of the fungus significantly decreased in both crowns and roots from the first sampling time to the second sampling time, while no significant decreases were recorded from the second to the third sampling time (Table 12). At the third sampling time, incidences of *Trichoderma* spp. recorded for the C (33.3%) and ECO (31.7%) treatments were significantly higher than the EXT (13.3%) and MF (11.7%) treatments. This could perhaps partly explain the significant difference in grain yield between these treatments. This confirms results obtained during the previous season (Lamprecht *et al*., 2006). Seventy representative isolates were characterised using molecular techniques. The species identified included *T. asperellum*, *T. hamatum*, *T. harzianum*, *T. koningiopsis* and *T. spirale*. It is, however, interesting that there were not significantly higher incidences of *Trichoderma* spp. recorded in crowns and roots of the Eco-T (ECO) treatment, although the application of Eco-T (*T. harzianum*) seemed to be responsible for the considerable increase in yield of this treatment compared to the results of the previous season.

Trichoderma spp. have been listed as both pathogens of maize and biocontrol agents for maize diseases (McFadden & Sutton, 1975; Elad, Zvieli & Chet, 1986; Srobarova & Eged, 2005). McFadden & Sutton (1975) showed that *T. koningii*, *T. harzianum* and *T. hamatum* can produce first internode lesions in maize seedlings. According to Hornby & Ullstrup (1967) and Whitney & Mortimore (1961) *Trichoderma* spp. occur frequently in senescent and dead maize tissues. The severity of damage to maize seedlings by *Trichoderma* spp. is influenced by the species composition and number of propagules of the fungus in the soil (McFadden & Sutton, 1975). The information on *Trichoderma* as a pathogen of maize is limited to Ontario, Canada, and it is uncertain to what extent *Trichoderma* spp. cause disease problems in other maize producing areas. It is, therefore, important that the different *Trichoderma* spp. that we isolated, should be evaluated for their ability to cause crown and root rot of maize in South Africa.

Table 10. Treatment effects on the incidence of fungal species in crowns and roots.

Fungus	PP[v]	Incidence (%)[wxy]															LSD[z]
		AN	BO	C	CAN	CR	ECO	EXT	MB	MF	N	OB	S	SF	SW	T	
Acremonium spp.	C	0.6a	1.7a	0.0a	0.6a	0.0a	0.6a	2.8a	0.0a	1.1a	0.0a	0.0a	0.6a	1.1a	1.1a	0.6a	NS
	R	0.0a	0.0a	0.0a	0.0a	0.0a	0.6a	1.1a	0.6a	0.0a	0.0a	0.0a	0.0a	0.6a	0.0a	0.0a	NS
F. equiseti	C	0.0a	0.0a	0.6a	0.0a	0.0a	0.0a	0.0a	0.0a	0.6a	0.6a	0.6a	0.0a	0.0a	0.6a	1.1a	NS
	R	Inter.	Inter.	Inter.	Inter.	Inter.	Inter.	Inter.	Inter.	Inter.	Inter.	Inter.	Inter.	Inter.	Inter.	Inter.	Inter.
F. graminearum	C	0.6cd	1.1cd	5.0ab	0.0cd	1.1cd	3.9a-c	6.7a	1.7b-d	1.7b-d	1.7b-d	1.1cd	2.8b-d	0.6cd	3.9a-c	0.6cd	3.47
	R	12.2e	23.9a-d	30.6a	11.7e	17.2c-e	25.6a-c	26.7a-c	17.8b-e	15.0de	27.2a-c	22.8a-d	27.8ab	13.9de	28.9a	11.1e	10.07
F. nygamai	C	1.1a	0.0a	0.0a	0.0a	0.0a	1.7a	0.0a	0.6a	1.1a	0.0a	0.0a	1.1a	0.6a	0.0a	1.7a	NS
	R	0.0a	1.1a	2.2a	3.8a	1.7a	1.7a	0.6a	1.7a	0.6a	2.2a	0.6a	1.7a	0.0a	0.6a	1.1a	NS
F. oxysporum	C	Inter.	Inter.	Inter.	Inter.	Inter.	Inter.	Inter.	Inter.	Inter.	Inter.	Inter.	Inter.	Inter.	Inter.	Inter.	Inter.
	R	32.2a	37.2a	16.1a	32.8a	30.0a	20.6a	21.1a	27.2a	35.6a	23.3a	19.4a	21.7a	26.7a	23.3a	30.6a	NS
F. proliferatum	C	1.1a	0.0a	0.0a	0.6a	2.2a	0.6a	0.6a	0.6a	4.4a	0.0a	0.6a	2.2a	0.0a	0.0a	0.6a	NS
	R	Inter.	Inter.	Inter.	Inter.	Inter.	Inter.	Inter.	Inter.	Inter.	Inter.	Inter.	Inter.	Inter.	Inter.	Inter.	Inter.
F. solani	C	0.0a	0.6a	0.6a	0.0a	0.0a	0.6a	0.0a	0.0a	1.1a	0.0a	0.0a	0.6a	0.0a	0.0a	0.6a	NS
	R	7.8a	3.9a	1.7a	2.2a	3.3a	2.8a	5.6a	5.0a	5.6a	1.1a	4.4a	5.0a	67a	3.9a	2.8a	NS
F. subglutinans	C	Inter.	Inter.	Inter.	Inter.	Inter.	Inter.	Inter.	Inter.	Inter.	Inter.	Inter.	Inter.	Inter.	Inter.	Inter.	Inter.
	R	1.7a	0.6a	1.1a	0.0a	1.7a	1.1a	0.0a	0.6a	1.1a	1.1a	1.1a	0.6a	1.1a	0.6a	1.1a	NS
Pyrenocheata	C	3.3a	1.1a	0.6a	1.1a	1.7a	3.9a	2.8a	0.0a	0.6a	1.7a	1.7a	0.0a	2.8a	0.0a	0.0a	NS

Fungus	PP[v]	Incidence (%)[wxy]															LSD[z]
		AN	BO	C	CAN	CR	ECO	EXT	MB	MF	N	OB	S	SF	SW	T	
Terrestris	**R**	10.0a	7.2a	8.9a	10.0a	7.8a	4.4a	8.9a	2.2a	6.7a	5.6a	11.1a	8.9a	8.9a	3.3a	5.6a	NS
***Pythium* spp.**	**C**	0.0a	0.0a	0.6a	0.6a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	NS
	R	0.6a	2.2.a	0.0a	0.6a	1.7a	1.7a	1.1a	0.0a	1.1a	0.0a	0.6a	1.7a	1.1a	0.0a	0.0a	NS
***Trichoderma* spp.**	**C**	2.2a	3.3a	16.1a	2.8a	5.6a	6.7a	4.4a	8.3a	10.6a	7.2a	5.6a	12.8a	5.0a	7.2a	8.9a	NS
	R	20.6a	25.6a	33.3a	21.7a	26.7a	28.3a	25.6a	38.3a	22.8a	28.3a	29.4a	29.4a	22.2a	26.1a	21.1a	NS

[v]PP = Plant Part; C = Crown; R = Root.

[w]Means within a fungus, within a plant part followed by the same letter do not differ significantly (P = 0.05).

[x]See Table 1 for treatment descriptions.

[y]Inter. = Indicates a significant treatment x sampling time interaction; See Table 11 for interaction means.

[z]LSD = Least significant difference at P = 0.05; NS = Not significant

Table 11. Interaction means for the effect of sampling time and treatments on the incidence of fungal species in crowns and roots.

Fungus	PP[v]	ST[w]	Incidence (%)[xy]															LSD[z]
			AN	BO	C	CAN	CR	ECO	EXT	MB	MF	N	OB	S	SF	SW	T	
F. equiseti	R	1	0.0g	1.7fg	0.0g	0.0g	1.7fg	0.0g	0.0g	0.0g	0.0g	0.0g	1.7fg	0.0g	1.7fg	0.0g	0.0g	3.75
		2	1.7fg	1.7fg	0.0g	1.7fg	0.0g	0.0g	0.0g	1.7fg	3.3e-g	0.0g	0.0g	0.0g	10.0a-c	0.0g	8.3b-d	
		3	1.7fg	0.0g	0.0g	3.3e-g	0.0g	0.0g	0.0g	11.7ab	13.3a	0.0g	0.0g	0.0g	5.0d-f	6.7c-e	3.3e-g	
F. oxysporum	C	1	3.3d-f	3.3d-f	0.0f	0.0f	3.3d-f	3.3d-f	5.0d-f	0.0f	13.3b-d	3.3d-f	3.3d-f	1.7ef	0.0f	1.7ef	3.3d-f	10.16
		2	6.7c-f	6.7c-f	5.0d-f	3.3d-f	10.0b-f	5.0d-f	1.7ef	6.7c-f	3.3d-f	3.3d-f	0.0f	6.7c-f	30.0a	3.3d-f	11.7b-e	
		3	1.7ef	1.7ef	10.0b-f	3.3d-f	0.0f	5.0d-f	5.0d-f	6.7c-f	8.3b-f	13.3b-d	5.0d-f	5.0d-f	5.0d-f	18.3b	16.7bc	
F. proliferatum	R	1	1.7bc	0.0c	0.0c	0.0c	1.7bc	0.0c	0.0c	1.7bc	13.3a	0.0c	0.0c	0.0c	0.0c	0.0c	1.7bc	3.72
		2	1.7bc	3.3bc	0.0c	0.0c	1.7bc	0.0c	0.0c	1.7bc	0.0c	0.0c	1.7bc	0.0c	0.0c	0.0c	0.0c	
		3	1.7bc	0.0c	0.0c	0.0c	0.0c	0.0c	0.0c	1.7bc	1.7bc	5.0b	0.0c	0.0c	0.0c	0.0c	0.0c	
F. subglutinans	C	1	0.0d	8.3a-c	0.0d	0.0d	1.7cd	0.0d	1.7cd	1.7cd	1.7cd	0.0d	0.0d	0.0d	1.7cd	0.0d	1.7cd	6.76
		2	0.0d	1.7cd	1.7cd	1.7cd	0.0d	0.0d	3.3b-d	1.7cd	3.3b-d	5.0b-d	10.0ab	3.3b-d	0.0d	0.0d	1.7cd	
		3	0.0d	1.7cd	5.0b-d	1.7cd	1.7cd	0.0d	0.0d	0.0d	3.3b-d	1.7cd	0.0d	0.0d	1.7cd	15.0a	5.0b-d	

[v]PP = Plant Part; C = Crown; R = Root.

[w]ST = Sampling time.

[x]Means within a fungus, within a plant part followed by the same letter do not differ significantly (P = 0.05).

[y] See Table 1 for treatment descriptions.

[z]LSD = Least significant difference at P = 0.05.

Table 12. Sampling time effects on the incidence of fungal species in crowns and roots of maize plants.

Fungus	Plant part	Incidence (%)^{wxy}			LSD^{z}
		ST1	ST2	ST3	
***Acremonium* spp.**	**Crown**	0.2 a	0.9 a	1.0a	NS
	Root	0.4 a	0.1 a	0.0a	NS
F. equiseti	**Crown**	0.0 a	0.3 a	0.4a	NS
	Root	Inter.	Inter.	Inter.	Inter.
F. graminearum	**Crown**	1.0 b	3.6 a	1.9ab	1.68
	Root	4.3 c	23.7 b	34.4a	4.30
F. nygamai	**Crown**	0.6 a	0.3 a	0.7 a	NS
	Root	1.7 a	1.4 a	0.8 a	NS
F. oxysporum	**Crown**	Inter.	Inter.	Inter.	Inter.
	Root	13.0 c	36.2 a	30.3 b	5.13
F. proliferatum	**Crown**	1.2 a	0.6 a	0.9 a	NS
	Root	Inter.	Inter.	Inter.	Inter.
F. solani	**Crown**	0.0 a	0.4 a	0.3 a	NS
	Root	1.3 c	6.7 a	4.3 b	2.10
F. subglutinans	**Crown**	Inter.	Inter.	Inter.	Inter.
	Root	0.1 b	1.3 a	1.2 a	0.98
Pyrenocheata Terrestris	**Crown**	0.9 a	1.4 a	1.9 a	NS
	Root	5.1 b 1	10.3 a	6.4 b	2.94
***Pythium* spp.**	**Crown**	0.0 a	0.1 a	0.1 a	NS
	Root	0.1 b	0.8 ab	1.6 a	0.98
***Trichoderma* spp.**	**Crown**	10.8 a	6.1 b	4.4 b	3.88
	Root	36.2 a	21.7 b	22.0 b	5.99

[w]ST = Sampling time.
[x]Means within a fungus, within a plant part followed by the same letter do not differ significantly (P = 0.05).
[y]Inter. = Indicates a significant treatment x sampling time interaction; See Table 11 for interaction means.
[z]LSD = Least significant difference at P = 0.05; NS = Not significant.

The second most prominent fungus obtained in this study was *F. oxysporum*. This is also similar to what was recorded during the previous season (Lamprecht *et al*., 2006). Although treatments did not significantly affect the incidences of the fungus on roots when averaged over sampling times, the fungus increased significantly on roots from the beginning to the end of the season (Table 12). When data for sampling times were analysed separately, there were significant treatment effects at the third sampling time, with significantly lower incidences of the fungus recorded for the ECO treatment compared to MF, SF and T treatments. The high incidences on some of the treatments that had low grain yields may point towards a more important role for *F. oxysporum* as a soilborne pathogen of maize than previously realized, but this needs to be investigated in future research. A significant (P = 0.0010) treatment x sampling time interaction was recorded for the incidence of the fungus on crowns of plants from the different treatments, showing significantly higher incidences of the fungus in crowns of the MF treatment compared to the C, CAN, MB, S, SF and SW treatments at the first sampling time, and significantly higher incidences for the SF treatment compared to the other treatments at the second sampling time. Significantly higher incidences were also recorded for the SW and T treatments compared to the AN, BO, CAN, CR, ECO, EXT, OB, S and SF treatments at the third sampling time (Table 11). *Fusarium oxysporum* did not significantly increase in crowns during the season for most of the treatments, however, incidences increased significantly for the SW and T treatments from the first to the third sampling time (Table 11). The incidences of this fungus were not significantly correlated with crown and root rot severity. It is also interesting to note that there was a significantly negative correlation (P = 0.0176, r = 0.60195) between the incidences of *F. oxysporum* and *F. graminearum*. This fungus was also previously recorded on maize roots in South Africa by Du Toit (1968) and Chambers (1987a,b) and more recently by Smit *et al*. (1997) on maize roots at Viljoenskroon. They also recorded high frequencies of the fungus and indicated that the fungus was isolated more frequently from discoloured than clean root tissue. *Fusarium oxysporum* is not regarded as an aggressive pathogen of maize. 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.

Fusarium graminearum was significantly affected by the treatments and sampling times in this study (Table 9). The highest incidences of the fungus in crowns were recorded for the EXT treatment and incidences on crowns of plants from this treatment differed significantly from those of the other treatments except for the C, ECO, and SW treatments. The lowest incidences in roots were recorded for the AN, CAN, CR, MB, MF, SF and T treatments (Table 10). In the previous study high incidences of *F. graminearum* was recorded for the MB treatment (Lamprecht *et al.,* 2006). This was not the case in this study where the incidences on roots of the ECO and EXT treatments did not differ significantly from the MB treatment and significantly higher incidences of the fungus were recorded on the control (C) treatment compared to the MB treatment. The fungus significantly increased in both crowns and roots from the first to the third sampling time (Table 12). Ramsey (1990) also reported higher incidences of *F. graminearum* after physiological maturity than earlier in the season. The fact that the incidences of the fungus were lower where canola (CAN), crambe (CR) and soyabean (SF) preceded the maize crop is an indication that crop rotation with non-host crops can be used in cases where this fungus poses a problem. It is, however, important to note that Pioli *et al*. (2004) recently showed that *F. graminearum* can infect soyabean pods and seeds and soyabean can, therefore, act as a "host" for this pathogen. Incidences of the fungus on black oats did not differ from the control (C) treatment. We could unfortunately not obtain any information on the susceptibility of black oats to *F. graminearum*. Tillage (T, MB and AN treatments) also seem to reduce the incidences of the fungus on roots. The incidences of *F. graminearum* on crowns and roots were significantly positively correlated with crown and root rot severity, especially at the third sampling time (crown rot – $P = 0.0075$, $r = 0.65957$; root rot – $P = 0.0112$, $r = 0.63384$). This strongly suggests that this fungus played an important role in crown and root rot severity in this study. The high incidences of *F. graminearum* in maize crowns and roots in our study are not unexpected, since wheat was the preceding crop for many of the treatments. Many researchers have found that *F. graminearum* increases under wheat-maize rotations (Schaafsma *et al*., 2005) The fungus is a serious pathogen causing scab of wheat and stalk and ear rot of maize (White, 1999) and can also cause seedling blight and root rot of maize (Du Toit, Kirby & Pedersen, 1997; Munkvold & O'Mara, 2002; Moreno-Gonzalez *et al*., 2004). *Fusarium graminearum* 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 did not evaluate the capacity of the fungus to cause root rot. Miller (1964) considers *F. graminearum* of prime importance as a soilborne pathogen of maize, but Hornby & Ullstrup (1967) only occasionally isolated *F. graminearum* from maize roots, and Lamprecht (2007) did not find it to be an important fungus associated with crown and root rot of maize at Vaalharts. The importance of the fungus as a crown and root rot pathogen under local conditions should be investigated in future research. It is also important to note that *F. graminearum* produces mycotoxins such as zearelenone and deoxynivalenol (Marasas, Nelson & Toussoun, 1984) and it was demonstrated that deoxynivalenol can act as a virulence factor (McCormick, 2003). There is, therefore, a close relationship between disease severity and DON concentration.

In this study, *P. terrestris* was significantly more frequently isolated from roots at the second compared to the first and third sampling times (Table 12). This confirms results obtained during the previous season (Lamprecht *et al.*, 2006). Treatments had no significant effect on the incidences of the fungus in crowns and roots, but it is interesting to note that the lowest incidences on roots were recorded for the MB treatment (Table 10). Young & Kucharek (1977) also isolated the fungus from maize seedlings and recorded maximum isolation frequency at the silking stage, but by full dent stage the fungus was no longer recovered. *Pyrenochaeta terrestris* was previously reported by Chambers (1987a) on maize roots in South Africa, but he did not conduct pathogenicity studies with the pathogen (Chambers, 1987b). Smit *et al.* (1997) obtained high numbers of *Phoma* spp. from maize roots, but it is uncertain whether these *Phoma* spp. included *P. terrestris*. Furthermore, there is no information available on the pathogenic potential of these *Phoma* spp. *Pyrenochaeta terrestris* is regarded as the primary pathogen in the complex causing red root rot of maize. Symptoms associated with this disease are a reddish pink discolouration of the roots and basal stalk tissue and the disease is not apparent until just prior to senescence. Red root rot occurs in many types of soil and the fungus survives well under a wide range of temperature and pH conditions (White, 1999).

Fusarium proliferatum was isolated from the roots of the AN, BO, CR, MB, MF, N, OB, and T treatments and from the crowns of all the treatments except the BO, C, N, SF and SW treatments (Tables 10 & 11). There was a significant (P = 0.0006)

treatment x sampling time interaction for the incidences of the fungus in roots. This showed that the fungus was significantly more frequently isolated from roots of the MF treatment at the first sampling time compared with the second and third sampling time, but there were no significant differences in the incidences of the fungus on roots of the other treatments at the three sampling times (Table 11). *F. proliferatum* is listed as an ear, stalk, seed and root rot pathogen, but the importance of this fungus as a maize pathogen is not clear (White, 1999). This fungus was also not previously associated with maize crown and root rot in South Africa. It is important to note that *F. proliferatum* can be easily confused with *F. verticillioides* (syn. *F. moniliforme*) and, since *F. proliferatum* was only described as a separate species in 1983, many researchers have identified *F. proliferatum* as *F. verticillioides* (White, 1999). *F. proliferatum* seems to be a less aggressive pathogen of maize (Munkvold & O'Mara, 2002) and it is possible that this fungus protected the plants from the more aggressive pathogens such as *Pythium* and *F. graminearum,* similarly to the protection of maize seedlings by *F. verticillioides* against *F. graminearum* (Van Wyk, Scholtz & Marasas, 1988).

A significant treatment x sampling time interaction was also recorded for the incidences of *F. subglutinans* in crowns ($P = 0.0253$) and *F. equiseti* in roots ($P < 0.0001$) of plants from the different treatments (Table 9). The highest incidences of the *F. subglutinans* were recorded at the second sampling time for the BO and the third sampling time for the SW treatments (Table 11). Treatments did not significantly affect the incidences of the fungus in roots, but the fungus increased on roots from the first to the third sampling time (Tables 10 & 11). Incidences of *F. equiseti* in roots increased significantly from the first to the third sampling time in roots from the MB and MF treatments, whereas the incidences of the fungus in roots of the SF and T treatments were highest at the second compared to the first and third sampling times (Table 11). Incidences of *F. equiseti* in crowns were not affected by treatments or sampling times (Table 10 & 11). *F. equiseti* was isolated by us from diseased crown and root tissues during the previous season (Lamprecht et al., 2006) and also previously from maize roots in South Africa by Smit *et al*. (1997). Low frequencies of the fungus were also recorded on maize roots by Warren & Kommedahl (1973) in the USA. *Fusarium equiseti* does not appear to be an important pathogen of maize. Similarly *F. subglutinans* is not listed as an important root rot pathogen of maize.

Sampling time significantly affected the incidences of *F. solani* and *Pythium* spp. in roots, but not crowns. These two fungi increased significantly in roots from the beginning to the end of the growth season (Table 12). *Pythium* spp. were also shown to increase in roots from the first to the third sampling time the previous season (Lamprecht *et al*., 2006). In the previous study, MB also reduced the incidences of *Pythium* spp. on roots significantly (Lamprecht *et al*., 2006). This was not the case in the present study and is most probably a result of the low incidences of *Pythium* spp. isolated due to drier conditions during the season compared to the previous season. *Fusarium solani* has been shown to reduce dry weights of maize significantly at high (29°C) temperatures, but appears to be a weak pathogen of maize (Warren & Kommedahl, 1973).

Incidences of *Acremonium* spp. in crowns and roots were not significantly affected by treatments and sampling times (Table 9, 10 & 11), however, the fungus increased in crowns from the first to the third sampling time during the previous season (Lamprecht *et al*., 2006). It should, however, be noted that *Acremonium* spp. were not as prevalent during this study as during the previous season. Incidences of *F. nygamai* were not affected by treatments or sampling times (Table 9). During the previous study, it was recorded that the incidences of the fungus decreased significantly in crowns from the first to the third sampling times (Lamprecht *et al*., 2006). Chambers (1987a, b) isolated *Acremonium* spp. from maize roots and demonstrated that these fungi did not cause significant reduction in seedling emergence, but there is no information on *F. nygamai* as a pathogen of maize.

Pythium spp. isolated in this study included *P. acanthicum*, *P. aristosporum*, *P. arrhenomanes*, *Pythium* HS-group, *P. irregulare, P. mamillatum*, *P. rostratifinges*, and *P. ultimum* var. *ultimum*. At least 14 *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. graminicola, P. irregulare, P. paroecandrum*, *P. pulchrum*, *P. rostratum*, *P. splendens*, *P. tardicrescens*, *P. ultimum* and *P. vexans* (White, 1999). Recent studies indicated that *P. arrhenomanes* is the primary cause of root rot of maize in the Midwestern United States (Deep & Lipps, 1996). 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). Relatively low frequencies of *Pythium* spp. were obtained in this study. This confirms results obtained during the previous season (Lamprecht *et al*., 2006). It was speculated at that stage that surface disinfestation may negatively impact on the isolation of *Pythium* spp. as previously reported by Denman *et al.* (1995) for lucerne. However, the fact that metalaxyl treatment of seed did not significantly affect survival and height of seedlings (see growth room study) showed that *Pythium* spp. are not important as pathogens of maize at Winterton early in the season.

Rhizoctonia spp. were infrequently isolated from crowns and roots in this study and isolates were identified to species and anastomosis group (AGs) level. The AGs obtained included *Rhizoctonia solani* AG-R and AG-2-2. *Rhizoctonia solani* AG 2-2IIIB and to a lesser extent *R. zeae* cause *Rhizoctonia* crown and brace root rot in France, Japan, New Zealand and the USA (White, 1999). The disease is more severe in irrigated intensively managed maize than in nonirrigated maize (Sumner & Dowler, 1983; White, 1999). Low incidences of *Rhizoctonia* spp. were previously obtained by Chambers (1987a,b) on maize in South Africa, but Rhizoctonia crown and brace root rot is currently not regarded as an important disease of maize locally.

Stubble coverage significantly affected the incidences of *F. equiseti* (roots), *F. graminearum* (crowns and roots), *F. oxysporum* (crowns and roots) and *F. proliferatum* (roots). *F. equiseti*, *F. oxysporum* and *F. proliferatum* were significantly more prevalent under no and partial cover than full cover, whereas the reverse was true for *F. graminearum* (Table 13). It, therefore, appears that the more virulent pathogens such as *F. graminearum* were favoured by more stubble coverage, and therefore conservation or no-till, compared to the weak pathogens, such as *F. equiseti, F. oxysporum* and *F. proliferatum.*

Table 13. Effect of stubble coverage on the incidence of fungal species in crowns and roots of maize plants.

Fungi	Plant Part	Stubble coverage^{xy}			LSD^{z}
		Full	**No**	**Partial**	
***Acremonium* spp.**	**Crown**	0.7 a	0.7 a	0.7 a	NS
	Root	0.2 a	0.3 a	0.0 a	NS
F. equiseti	**Crown**	0.3 a	0.4 a	0.0 a	NS
	Root	0.4 b	4.9 a	1.1 b	1.64
F. graminearum	**Crown**	3.6 a	1.1 b	0.7 b	1.61
	Root	27.1 a	14.4 b	16.3 b	4.66
F. nygamai	**Crown**	0.4 a	1.0 a	0.3 a	NS
	Root	1.3 a	0.8 a	1.7 a	NS
F. oxysporum	**Crown**	5.0 b	8.8 a	3.6 b	3.06
	Root	20.8 b	30.0 a	33.6 a	6.91
F. proliferatum	**Crown**	0.6 a	1.4 a	1.0 a	NS
	Root	0.3 b	1.8 a	1.0 ab	1.19
F. solani	**Crown**	0.2 a	0.4 a	0.1 a	NS
	Root	3.5 a	5.0 a	4.3 a	NS
F. subglutinans	**Crown**	2.2 a	1.9 a	1.5 a	NS
	Root	0.8 a	1.0 a	1.0 a	NS
Pyrenocheata	**Crown**	1.5 a	0.8 a	1.8 a	NS
terrestris	**Root**	7.3 a	5.8 a	8.8 a	NS
***Pythium* spp.**	**Crown**	0.1 a	0.0 a	0.1 a	NS
	Root	0.7 a	0.6 a	1.3 a	NS
***Trichoderma* spp.**	**Crown**	8.6 a	8.2 a	3.5 ab	4.45
	Root	28.7 a	26.1 a	23.6 a	NS

^{x}Stubble coverage: Full = C, ECO, EXT, N , OB, S, SW; Partial = AN, , BO, CAN, CR; No =,MB, MF, SF, T.
^{y}Means within a fungus, within a plant part followed by the same letter do not differ significantly (P = 0.05).
^{z}LSD = Least significant difference at P = 0.05.

A considerable amount of information is available on the effects of conservation tillage on ear and stalk rot pathogens of maize. In South Africa, Flett, McLaren & Wehner (1998) reported that mouldboard plough plots consistently had lower incidences of Stenocarpella ear rot than reduced tillage practices. However, Smit (1998) concluded that the effect of tillage practices on soilborne pathogens of maize were inconsistent in trials that she conducted at Bloekomspruit (Gauteng province) and Mmabatho (North West province). Sumner *et al*., (2002) in the USA reported that conservation tillage can increase Rhizoctonia crown and brace root rot of maize, and according to Scott (1993) minimum tillage promotes black root rot (*Pythium* spp.) in South Africa. It was also suggested by Deep & Lipps (1996) that *P. arrhenomanes* is favoured by poorly drained soil when continuous maize cropping and no-till are practised. Crop rotation is very important in no-till maize production systems. Howard (1998) found that maize yields were increased 14% and soyabean yields 11% with rotations in no-till systems. Since conservation tillage is increasingly promoted in South Africa, it is of utmost importance to determine the combined effect of crop rotation and conservation tillage on soilborne diseases of maize in order to reduce yield losses due to these diseases to a minimum.

Crop rotation and tillage can significantly impact on soilborne diseases. Crop rotation of maize with crops such as soyabean has been shown to significantly increase yield of maize (Lipps & Deep, 1991). In this study the grain yields of maize rotated with canola (CAN), crambe (CR) and black oats (BO) were higher than maize rotated with a fallow after soyabean (SF). Although crop rotation is recommended as a control measure against soilborne pathogens of maize and small grains (Williams & Schmitthenner, 1963), information on the effect of crop rotation on soilborne diseases of maize is limited. In South Africa, Smit *et al*. (1997) studied the effect of monoculture maize and rotation with soyabean, sunflower and groundnut on the incidence of maize root rot. According to them the effect of crop rotation was inconsistent and they concluded that “crop rotation may have a long-term effect on soil fungus populations which may only be evident after a longer period of time”. Kruger & Speakman (1997) in Germany, found that monoculture maize led to a high level of root rot after about 4 years, whereas disease levels were lower in rotations, even those containing a high proportion of cereals. It is well known that rotation of maize with wheat results in an increase in the incidence of scab on wheat and ear and

stalk rot of maize (Schaafsma *et al*., 2005). In our study, many of the treatments were applied when maize followed wheat and this resulted in high incidences of *F. graminearum* in roots. Since this fungus is regarded by certain researchers as an aggressive pathogen on maize roots (Miller, 1964), future studies should investigate the effect of other rotations on the incidence of this fungus on maize roots, but also other soilborne pathogens of importance to maize.

Incidence of nematodes in roots and soil

Nematode communities in crop soils are highly variable in their composition. Populations and community composition are influenced by many factors including the plant and microbe communities as well as crop residues in the soil (Osborne *et al.,* 2004). Predacious, fungiphagous and bacteriophagous nematodes were counted together as non-parasitic nematodes, because they play an important role in soil fertility and agricultural productivity. The composition of nematode communities (plant parasitic and free-living) correlates well with nitrogen cycling and decomposition, two of the most critical ecological processes in soil. Moreover, free-living nematodes are killed easily by relatively low doses of chemicals and are, therefore, useful indicative organisms of pollution in soil and also the general soil health. The incidences of non-parasitic nematodes on the roots and soil at the first sampling time differed significantly, with the highest incidence in roots recorded in the AN treatment and the highest incidence in the soil recorded in the C treatment (Tables 15 & 16). The general rule in cultivated soils for the percentage of plant-parasitic nematodes to non-parasitic nematodes is about 33 % to 66 % (Griffiths *et al.,* 1994; Yeates & Bongers, 1995). These percentages were only recorded in the MB treatments.

Table 15. Treatment effects on the incidence of nematode species in soil.

Nematode sp.[u]	ST	Incidence[v,w]															LSD[x]
		AN	BO	C	CAN	CR	ECO	EXT	MB	MF	N	OB	S	SF	SW	T	
Non-plant parasitic	**1**	348.3[y]a	476.7a	860a	515.0a	531.7a	451.7a	629.0a	292.5a	333.3a	390.0a	838.3a	653.3a	268.3a	808.3a	576.7a	NS
	3	325.0a	175.0a	460.0a	355.0a	205.0a	505.0a	173.3a	191.7a	261.7a	190.0a	721.7a	505.0a	213.3a	848.3a	303.3a	NS
Pratylenchus brachyurus, Pratylenchus zeae[u]	**1**	90.0a	11.7a	70.0a	73.3a	123.3a	55.0a	131.7a	2.5a	373.3a	58.3a	108.3a	110.0a	176.7a	103.3a	238.3a	NS
	3	558.3a	413.3a	506.7a	831.7a	690.0a	406.7a	431.7a	5.0a	288.3a	608.3a	881.7a	358.3a	550.0a	586.7a	158.3a	NS
Helicotylenchus spp./Scutellonema brachyurus	**1**	450.0de	1308.3bc	1516.7ab	2235.0a	1515.0ab	1603.3ab	1645.0ab	32.5e	1398.3b	973.eb-d	1225.0b-d	1381.3bc	1638.3ab	1670.0ab	546.7c-e	847.87
	3	4743a	4415.0a	7108a	8137.0a	4965a	4265.0a	7677.0a	87.0a	5520.0aa	4187.0a	8972.0a	6468a	8045.0a	8735.0a	1403.0a	NS
Rotylenchulus parvus	**1**	56.7a	50.0a	75.0a	206.7a	213.3a	75.0a	63.3a	2.5a	53.3a	28.3a	96.7a	115.0a	131.7a	133.3a	48.3a	NS
	3	178.3a	128.3a	546.7a	415.0a	140.0a	475.0a	580.0a	3.3a	1530.0a	300.0a	1060.0a	318.3a	1401.7a	710.0a	211.7a	NS
***Meloidogyne* spp.**	**1**	0.0a	0.0a	0.0a	3.3a	0.0a	1.7a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	NS
	3	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	1.7a	83.3a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	15.0a	NS
Criconemoides sphaerocephalus	**1**	6.7a	1.7a	1.7a	6.7a	1.7a	3.3a	1.7a	2.5a	5.0a	0.0a	5.0a	0.0a	0.0a	3.3a	1.7a	NS
	3	5.0a	33.3a	0.0a	13.3a	43.3a	8.3a	15.0a	1.7a	46.7a	0.0a	8.3a	16.7a	0.0a	0.0a	3.3a	NS
Hemicycliophora sp.	**1**	-[z]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	3	0.0a	20.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	NS
Paratrichodorus minor	**1**	0.0a	21.7a	8.3a	120.0a	5.0a	3.3a	6.7a	0.0a	6.7a	6.7a	10.0a	15.0a	26.7a	1.7a	3.3a	NS
	3	8.3a	151.7a	23.3a	28.3a	50.0a	63.3a	30.0a	3.3a	26.7a	3.3a	43.3a	15.0a	16.7a	40.0a	6.7a	NS
Longidorus pisi	**1**	0.0a	3.3a	23.3a	5.0a	10.0a	3.3a	1.7a	0.0a	0.0a	6.7a	3.3a	1.7a	3.3a	1.7a	13.3a	NS
	3	13.3bc	13.3bc	6.7bc	8.3bc	11.7bc	25.0a-c	0.0c	0.0c	30.0a-c	16.7bc	28.3a-c	10.0bc	58.3a	41.7ab	0.0c	NS

[u]Nematodes recorded in taxonomical order (Table 17)

[v] Nematodes were extracted from 250 g soil; Means in a row followed by the same letter do not differ significantly (P = 0.05)

[w]See Table 1 for treatment abbreviations

Table 16. Treatment effects on the incidence of nematode species in roots.

Nematode sp. [u]	Sampling time	Incidence [vw]															LSD [x]
		AN	BO	C	CAN	CR	ECO	EXT	MB	MF	N	OB	S	SF	SW	T	
Non plant parasitic	1	422.3[y]a	271.7a	280.3a	53.3a	121.3a	172.0a	406.7a	154.0a	229.3a	126.7a	244.0a	364.3a	323.7a	301.0a	29.7a	NS
	3	120.0a	221.7a	215.0a	118.3a	150.0a	85.0a	216.7a	223.3a	121.7a	126.7a	271.7a	195.0a	130.0a	161.7a	243.3a	NS
Pratylenchus brachyurus, Pratylenchus zeae[u]	1	402.7be	237.7c-e	367.3c-e	206.3de	906.3ab	175.0e	300.3c-e	5.7e	714.3a-c	500.0a-e	400.7c-e	158.0e	913.7a	697.7a-d	23.7e	504.41
	3	746.7a	463.3a	1266.7a	445.0a	1620.0a	231.7a	850.0a	30.0a	661.7a	433.3a	785.0a	565.0a	691.7a	323.0a	686.7a	NS
Helicotylenchus spp./Scutellonema brachyurus	1	51.0a	223.0a	211.0a	461.0a	380.7a	187.0a	470.3a	10.3a	294.7a	219.3a	375.0a	257.7a	426.7a	556.7a	107.7a	NS
	3	220.0a	221.7a	815.0a	411.7a	363.3a	305.0a	336.7a	156.7a	195.0a	443.3a	91.7a	295.0a	277.0a	313.3a	208.3a	NS
Rotylenchulus parvus	1	63.7bc	168.7bc	64.3bc	64.0bc	54.0bc	229.3b	42.7bc	2.7c	101.3bc	48.7bc	18.3c	52.0bc	426.0a	143.3bc	4.7c	194.36
	3	6.7a	33.3a	166.7a	48.3a	81.7a	153.3a	45.0a	0.0a	53.3a	35.0a	68.3a	95.0a	55.0a	81.7a	56.7a	NS
Meloidogyne spp.	1	4.3a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	NS
	3	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	18.3a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	NS
Criconemoides sphaerocephalus	1	0.0a	0.0a	0.0a	0.0a	3.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	6.0a	3.7a	0.0a	NS
	3	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	1.7a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	NS
Hemicycliophora sp.	1	-[z]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	_
	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Meloidogyne spp.	1	4.3a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	NS
	3	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	18.3a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	NS
Paratrichodorus minor	1	0.0a	0.0a	65.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	2.7a	2.7a	1.7a	NS
	3	0.0a	0.0a	5.0a	0.0a	0.0a	3.3a	0.0a	0.0a	0.0a	0.0a	0.0a	3.3a	6.7a	0.0a	0.0a	NS
Longidorus pisi	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

[u] Nematodes recorded in taxonomical order (Table 17)
[v] Nematodes were extracted from 20 g roots; Means in a row followed by the same letter do not differ significantly (P = 0.05)
[w] See Table 1 for treatment abbreviations
[x] NS – not significant
[z] Not isolated from roots

The feeding of plant-parasitic nematodes on plant tissue, their interaction with fungi and bacteria or their transmission of viruses can significantly influence the growth and reduce the yield of crops. Crops susceptible to nematodes may allow a quick build-up to high nematode population numbers (Keetch & Milne, 1982). Even though we will report on each nematode genus separately, it is important to note that endoparasitic, semi-endoparasitic and ectoparasitic nematodes act as a complex and that concomitant pathogen interactions do occur. One hundred and sixteen plant-parasitic nematode species belonging to 24 genera are reported to be associated with maize in South Africa (Kleynhans et al., 1996; South African Plant-Parasitic Nematode Survey database). All the nematodes that have been found at the trial site (Table 17) have previously been reported in association with maize in South Africa.

Table 17. Plant-parasitic nematodes found in the soil and roots during the first and third sampling.

		First sampling	Third sampling
	Common Name	**% incidence [y]**	**% incidence [z]**
1. Pratylenchidae			
Pratylenchus brachyurus (Godfrey, 1929) Filip'ev & Schuurmans Stekhoven, 1941	Lesion nematode	19	35
Pratylenchus zeae Graham, 1951	Lesion nematode	84	93
2. Hoplolaimidae			
Helicotylenchus digonicus *Perry in Perry, Darling & Thorne, 1959*	Spiral nematode	-	2
Helicotylenchus dihystera (Cobb, 1893) Sher, 1961	Spiral nematode	96	98
Helicotylenchus paraplatyurus *Siddiqi, 1972*	Spiral nematode	2	-
Helicotylenchus pseudorobustus *(Steiner, 1914) Golden, 1956*	Spiral nematode	8	-
Scutellonema brachyurus *(Steiner, 1938) Andrássy, 1958*	Spiral nematode	96	98
Rotylenchulus parvus (Williams, 1960) Sher, 1961		90	84

	Common Name	First sampling % incidence [y]	Third sampling % incidence [z]
3. Heteroderidae			
Meloidogyne ***spp. (immature)***	Root-knot nematode	4	-
Meloidogyne incognita (Kofoid & White, 1919) Chitwood, 1949	Root-knot nematode	-	2
Meloidogyne javanica (Treub, 1885) Chitwood, 1949	Root-knot nematode	2	9
4. Criconematidae			
Criconemoides ***spp. (immature)***	Ring nematode	15	-
Criconemoides sphaerocephalus ***Taylor, 1936***	Ring nematode	15	17
Hemicycliophora spp. (immature)	Sheath nematode	-	1
5. Trichodoridae			
Paratrichodorus minor ***(Colbran, 1956) Siddiqi, 1974***	Stubby-root	44	44
6. Longidoridae			
Longidorus pisi ***Edward, Misra & Singh, 1964***	Needle	33	35

[y]Percentage incidence in total number of samples collected during the first sampling
[z] Percentage incidence in total number of samples collected during the third sampling

In a questionnaire survey, South African nematologists regarded *Pratylenchus* as the second most economically important genus after *Meloidogyne* in South Africa (Keetch, 1989). *P. brachyurus* and *P.* zeae were the most common endoparasites found at the trial site. These nematodes are common in maize fields and are often associated with poor growth (McDonald & Nicol, 2005). In Zimbabwe, Martin *et al*. (1975) found that a good host plant such as maize will permit an increase in the *Pratylenchus brachyurus* population, which alone could produce a yield loss of approximately 25 %. *P. zeae* causes greater mechanical breakage of cells but less necrosis and stunting of root growth than *P. brachyurus* and usually outnumber *P. brachyurus* when they do occur together (De Waele & Jordaan, 1988). According to Olowe & Corbett (1976) *P. zeae* dominates *P. brachyurus* in maize roots because of its shorter life cycle, faster reproductive rate and higher tolerance for a wider range of temperature. At the first sampling the highest incidences of *P. brachyurus* and *P. zeae* in roots were recorded for the CR and SF treatments (Table 16). High numbers were also recorded at the third sampling time for the

two fallow (MF & SF) treatments. It is interesting to note that the two fallow treatments, on which initial growth was good, deteriorated markedly as the season advanced. Todd (1991) reported that attempts to control lesion and sting nematodes (*Belonolaimus* spp.) in maize fields by fallowing have not been successful. When maize followed one year of fallow, the lesion and sting nematodes increased to densities equal to areas planted in continuous maize. At the third sampling time the incidences were highest in the C and CR treatments with a significant increase from the first to the third sampling time for the C treatment. The lowest incidences were recorded at the first sampling time for the MB and T treatments and at the third sampling for the MB treatment. Walters (1979) reported that MB fumigation to control *P. zeae* gave a substantial increase in maize yields. The highest incidences of lesion nematodes in the soil were recorded for the MF and T treatments at the first sampling time and the lowest incidences for the BO and MB treatments. At the third sampling time, the highest incidences of *P. brachyurus* and *P. zeae* were recorded in soil from the CAN, CR, and OB treatments and the lowest incidences from the MB treated soil. There was also an increase in the incidences of lesion nematodes in the roots from the first to the third sampling times. Interestingly, according to Inomoto *et al.* (2006) the use of black oats (BO) as an autumn or winter crop has not affected the soil population of *P. brachyurus.*

The semi-endoparasitic genus *Helicotylenchus* and ectoparasitic genus *Scutellonema* are extremely common in South African soils (Kleynhans *et al*., 1996). The highest population numbers were recorded for these genera at both the first and third sampling times. The population numbers of *Helicotylenchus* spp. and *S. brachyurus* increased in the soil from the first sampling time to the third sampling time from all the treatments. At the third sampling time the population numbers of these nematodes reached very high levels. Population levels of 72 individuals' g^{-1} soil were recorded from the SF treatment. The threshold density for *H. dihystera* on maize in field microplots was found to be more than 2 individuals g^{-1} soil (Windham, 1998). The same trend of very high population numbers was also found in a survey of a tillage trial in the Eastern Cape Province (Marais & Swart, 2007). Trials conducted in Kenya to test the influence of nematodes in improved fallow practices showed that populations of root-knot nematodes were drastically reduced, but both maize and beans experienced heavy losses on soil under improved fallow probably due to a high

number of spiral nematodes, which became dominant in the nematode community (Kandji *et al*., 2003). Maize plants attacked by spiral nematodes may have numerous small light to dark brown lesions on their roots (Taylor, 1961). The highest incidence of these nematodes in roots at the first sampling time were from plants from the CAN, EXT, SF and SW treatments and the lowest incidence from the AN and MB treatments (Table 16). The markedly lower counts of these nematodes on roots in comparison to that found in the soil is expected, as these spiral nematodes (Table 17) are ectoparasites and semi-endoparasites. *Helicotylenchus* spp. and *S. brachyurus* were more frequently obtained from soil in the C, CAN, EXT, OB, S, SF and SW treatments during the first sampling time. The highest incidence of all the different plant-parasitic nematodes were found in soil from the CAN, OB, SF and SW treatments at the third sampling time (Table 15). *Rotylenchulus parvus* was recorded from most of the treatments (Table 17). De Waele & Jordaan (1988) also reported *R. parvus* from most of the maize fields sampled during the 1984/85 growing season. The high incidence of *R. parvus* supports previous findings that maize is a good host of this nematode (Shepherd, 1977; Van den Berg, 1978; De Waele & Jordaan, 1988). *R. parvus* was significantly more frequently obtained from roots from the SF treatment at the first sampling time. The highest incidence of *R. parvus* in the soil was for the MF treatment at the third sampling time (Table 15). There was a significant increase in the incidences of *R. parvus* from the first to the third sampling time. Furstenberg & Heyns (1975) also reported a great quantative increase in the soil. In an experiment at Rietondale Research Station, Pretoria, *R. parvus* initially made up 16 % of the total plant-parasitic nematodes but at the end of the experiment the population number had increased to 90 % of the nematode population.

Meloidogyne incognita and *Meloidogyne javanica* have been detected damaging maize in almost all maize-growing regions of the world (McDonald & Nicol, 2005). The typical gall-forming symptoms usually associated with root-knot nematodes may be totally absent. Maize was therefore often mistakenly seen as a poor host and even immune to root-knot nematodes (Riekert, 1996a). Concomitant pathogen interaction between root-knot nematodes and other pathogens has been observed and documented in both greenhouse and field experiments. As early as 1892, Atkinson reported that the severity of *Fusarium* wilt of cotton was increased in the presence of root-knot nematodes (Atkinson, 1892). In greenhouse trials, maize root and shoot growth were

more affected in pots infected with both *Fusarium moniliforme* and *M. incognita* than in pots infected with either pathogen alone (Palmer & MacDonald, 1974). Incidence of root-knot nematodes in the roots and soil were affected by treatments at the third sampling (Tables 15 & 16). The incidence of root-knot nematodes increased in the roots and soil from the first to third sampling time in the MB treatment. A possible explanation for this phenomenon is the vertical distribution and movement of these nematodes through the soil to an empty niche (Been & Schomaker, 2006).

Maize is an excellent host for *Criconemoides* spp. or ring nematodes**,** but**,** although huge increases in nematode numbers on maize have been observed over a growing season, it has been difficult to establish pathogenecity of these nematodes on maize (Johnson, 1975; Barker *et al.*, 1982; Windham, 1998). The highest incidences of this nematode were recorded in the soil from the BO, CR and MF treatments. This nematode also significantly increased in the soil from the first to the third sampling time for the BO, CR and MF treatments (Table 15).

Paratrichodorus minor or stubby root nematode is among the most common ectoparasite associated with maize in South Africa (De Waele & Jordaan, 1988). This nematode is also considered an important parasite of maize in the USA (Windham, 1998; Jackson, 2005). At the first sampling time the highest incidence of *P. minor* in roots was recorded for the C treatment and in the soil for the CAN treatment (Tables 15 & 16). The incidence of the stubby-root nematode in the roots for the SF treatment increased from the first to the third sampling time. Incidences of *P. minor* were higher in soil of the BO treatment compared to the other treatments at the third sampling time, and there was also an increase in the incidence of this species for most of the treatments from the first to the third sampling time.

During a survey of South African maize fields, De Waele & Jordaan (1988) found *Longidorus pisi* or needle nematode in most of the fields. Needle nematodes have only recently been shown to be pathogens of maize (Windham, 1998). The root injury caused by *L. brevilineatus* in the USA **r**esembles damage caused by *Paratrichodorus* spp. (Windham, 1998). Interestingly, on cotton and Jew's mallow, the stunting of shoots and poor root development have been associated with *L. pisi* (Aboul-Eid, 1972;

Yassin, 1974). The incidence of *L. pisi* was the highest in soil from the SW treatment than soil from the EXT, MB and T treatments at the third sampling time.

According to Inomoto *et al.* (2006), even though no till has been extensively used in Brazil and the USA, there is very little known about the effect of no till on plant-parasitic nematode populations. There is also very little known about the impact of conservation tillage on nematode populations in South Africa and, furthermore, the reported impact of tillage practices on nematode populations has been inconsistent (Barker & Koennig, 1998). In both Nebraska and Illinois reduced tillage, minimal or no-till in maize production are considered as risk factors in managing plant-parasitic nematodes (Jackson, 2005; Niblack, 2007). According to McSorley & Gallaher (1993), the population numbers of lesion nematodes were higher in conventionally tilled maize plots compared to minimum-till or no-till plots. Bergeson & Ferris (1986) suggested that no-till plots had more compact soil, which would limit root growth and restrict nematode movement, but in some cases lesion and also spiral nematodes have been less in conventionally tilled plots than in no-till plots (Windham, 1998). This could possibly be attributed to the fact that in conventionally tilled fields the nematodes are exposed to adverse environmental conditions (Pankaj *et al.,* 2006). At both the first and third sampling time the incidences of the plant-parasitic nematodes were lower in the tilled (T) plots than in the C and SW plots. This is especially marked in the case of the spiral nematodes, where high numbers were recorded at both the C and SW treatments during the third sampling time.

One of the key aspects in control or management of nematodes is the prevention of build-up of plant-parasitic nematode populations to critical levels (Riekert, 1996b; Haycock *et al.,* 2006), because yield loss is normally a response to damage to the root system of a maize plant (Du Plessis, 2003). A possible explanation for the failure of the nematicide treatment in this trial is that the application applied at planting did not adequately penetrate the heavy textured soil. However, this seems unlikely, as the soil is extremely well drained and 15 mm of irrigation was applied immediately after application of the nematicide. A number of reports also exist about the inconsistent yield reaction experienced with chemical control of plant-parasitic nematodes (McDonald & De Waele, 1987; McDonald *et al*., 1987*:* Riekert, 1996b), but when

infestation levels are high, chemical control in irrigated maize is still recommended (Du Plessis, 2003).

According to the analysis of the first and third sampling, none of the fallow or rotation treatments showed promising results. The spiral nematodes (*Helicotylenchus* spp. and *S. brachyurus*) are able to enter anhydrobiosis (Demeure *et al.,* 1979). Anhydrobiosis is a state of suspended animation characterized by nearly complete loss of all water. Organisms in anhrydobiotoc condition will revive after rehydration (Eisenback, 1998). Anhydrobiosis also occurs with *Pratylenchus thornei* and *Pratylenchus penetrans* (Glazer & Orion, 1983; Townshend, 1987). According to De Waele & Jordaan, (1988) it is possible that the same mechanism enables *P. zeae* and *P. brachyurus* in South Africa to survive the almost six months of drought between the two maize growing seasons. According to Todd (1991), attempts to control lesion and sting nematodes in maize fields by fallowing have not been successful. When maize followed one year of fallow, the lesion and sting nematodes increased in densities equal to areas planted to continuous maize and even though nematode populations decreased rapidly in fallowed plots, nematodes were still at detectable levels after 2 years without a cultivated crop (Windham, 1998). A long rotation of up to three or four years between susceptible crops, depending on the nematode species present, will give maximum increase in yield (Bridge, 1987). None of the plant-parasitic nematodes that have been found are host specific and will be able to survive and replicate on wheat and soyabean (Kleynhans *et al*., 1996) and, therefore, maintain high population numbers.

Functional diversity of soil microbial populations

Community level physiological profiles. A wide variety of compounds are released by plant roots into the surrounding soil. These compounds, e.g. carbohydrates, amino acids, organic acids, vitamins, polysaccharides and enzymes, create unique environments for the microorganisms living in the rhizosphere (Garbeva *et al*., 2004). During the pre-plant sampling, a distinguishable dissimilarity could be observed between carbon source utilisation patterns of soil microbial populations under no-cover (MF, SF, MB, T), full-cover (C, N, SW) and partial-cover (CR, BO, CAN)

treatments (Fig. 15). The MB and T treatments had been subjected to tillage, but the others had not. The carbon source utilisation patterns of soil microbes changed slightly since the previous season.

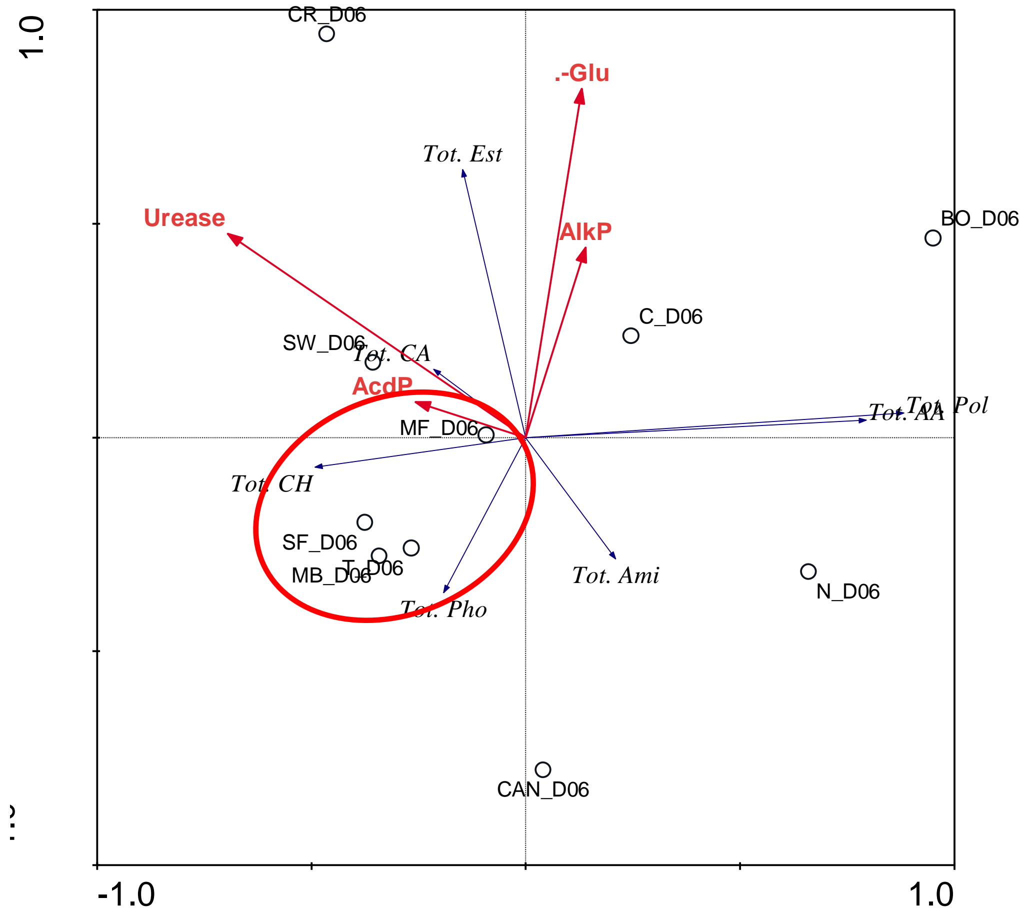
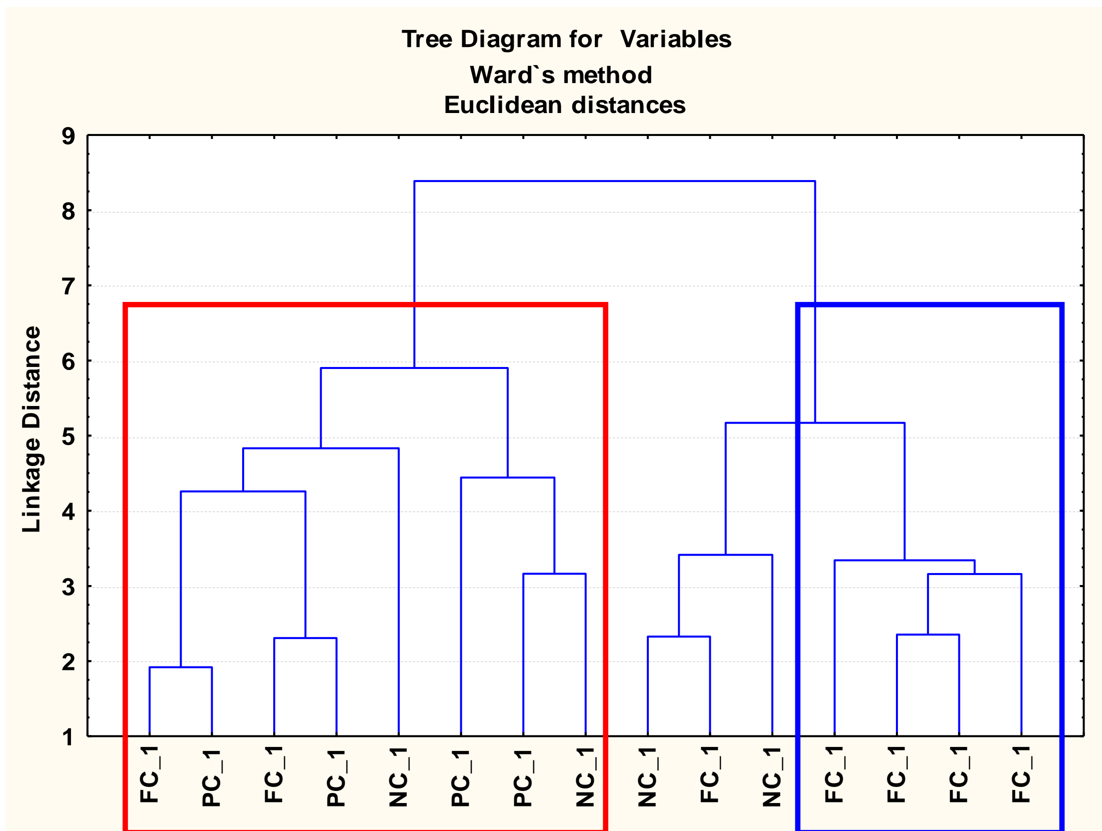


Fig. 15. A redundancy analysis ordination triplot illustrating distinguishable difference in carbon source utilisation patterns of different cover plots during pre-plant sampling.

Soil microbial carbon source utilisation patterns changed from pre-plant sampling to the first sampling following the planting of maize. Although no meaningful statistical difference existed between the three cover treatments (results not shown), a visible distinction could be made between carbon source utilisation in full-cover (FC) treatments compared to the other treatments (Fig. 16).



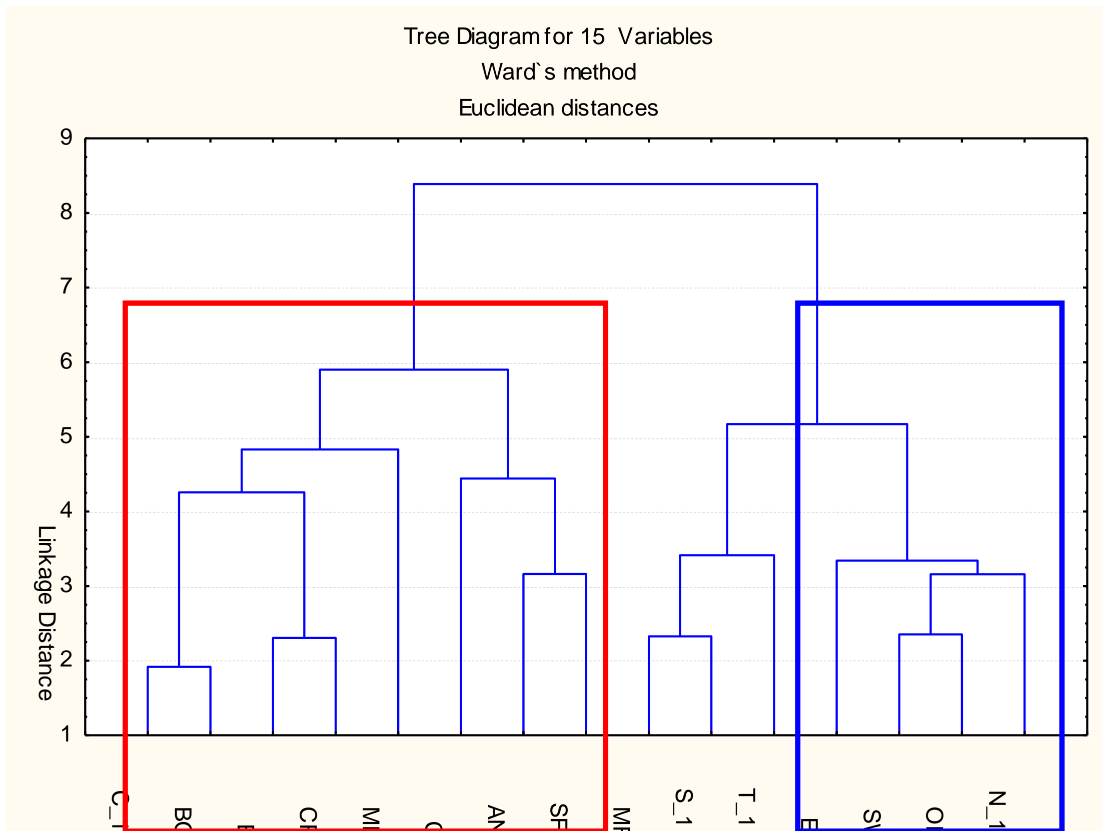


Fig. 16. **Dendogram illustrating the differences in carbon source utilisation patterns between the full-cover (FC), no-cover (NC), and partial-cover (PC) treatments.**

A redundancy analysis (RDA) ordination triplot illustrating the relationship between treatments, enzyme activity and carbon source utilisation profiles during the first and third sampling is presented in Figure 17.

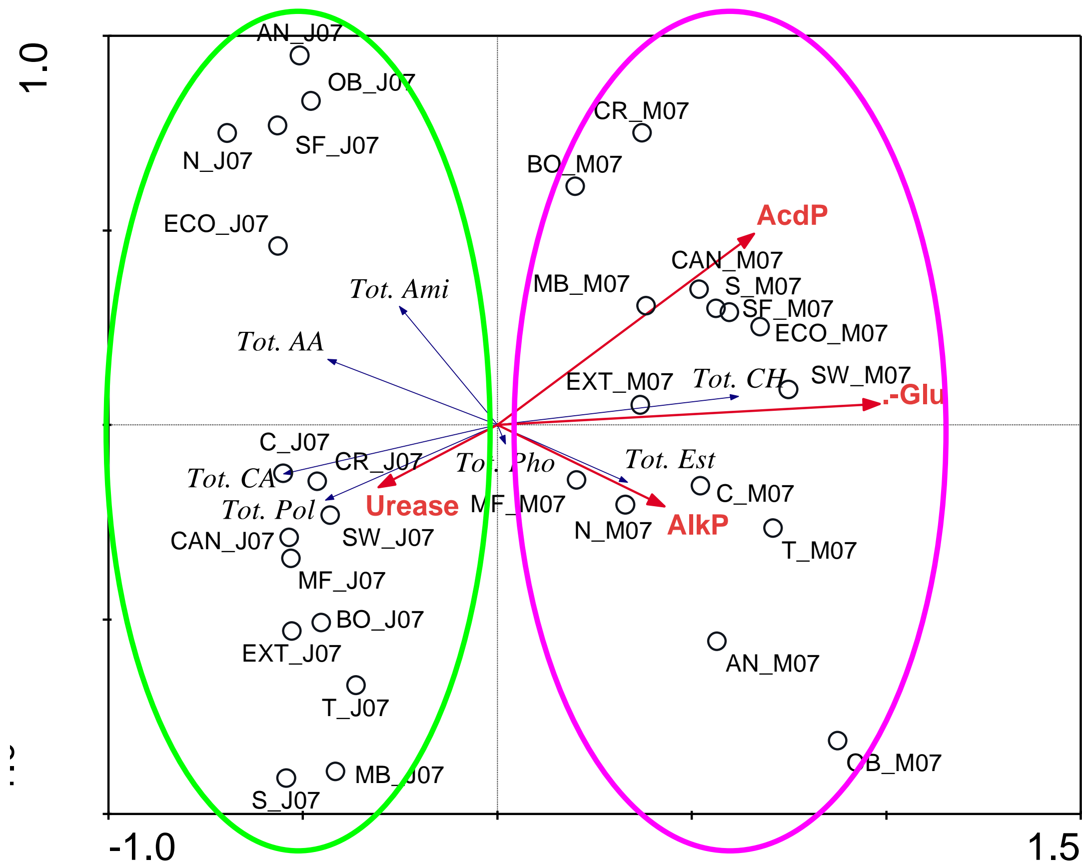


Fig. 17. **A Redundancy analysis ordination triplot illustrating the difference in carbon source utilisation profiles of cover treatments plots during first sampling (green) and third sampling (pink).**

Root exudate composition changes as maize seedlings mature, due to different nutritional requirements through different growth stages (Garland, 1996). Soil microbial populations in the rhizosphere also change accordingly, depending on their ability to utilise specific carbon sources (Garbeva *et al.*, 2004). Soil microbial populations mainly utilised amino acids, amides and carboxylic acids during the first sampling. Catabolisation of these carbon / nitrogen groups were confirmed by a higher urease enzyme activity in the soil, in comparison to soil samples taken during the third sampling. The latter samples positively correlated with β-glucosidase, acid phosphatase and alkaline phosphatase activity. β-glucosidase activity positively correlated with carbohydrate-presence in soil samples taken during the third sampling.

A strong positive correlation was also found between carboxylic acids utilisation and root rot during the third sampling (results not shown).

***Biodiversity indices*.** Shannon-Weaver substrate diversity indices distinguish soil populations based on the number of different carbon sources utilized. According to Magurran (1988), values of the index range between 1.5 and 3.5 and rarely increase above 4.5. Diversity index values obtained from the different treatments between the pre-plant sampling time and the third sampling time, were within the intermediate diversity range (2.6 – 2.9). This diversity range is similar to results obtained during the previous season at Winterton (Lamprecht *et al*., 2006).

Fall-off in soil microbial diversity towards the end of the season can be attributed to carbohydrate withdrawal by the cob, as well as plant maturity, resulting in a nutrient reduction in the rhizosphere (Fig. 18). The no-cover biodiversity initiated on the lowest level, but all treatments returned to the same level by the third sampling. This is indicative of a "stable" soil microbial population with the ability to cope with any soil disturbances.

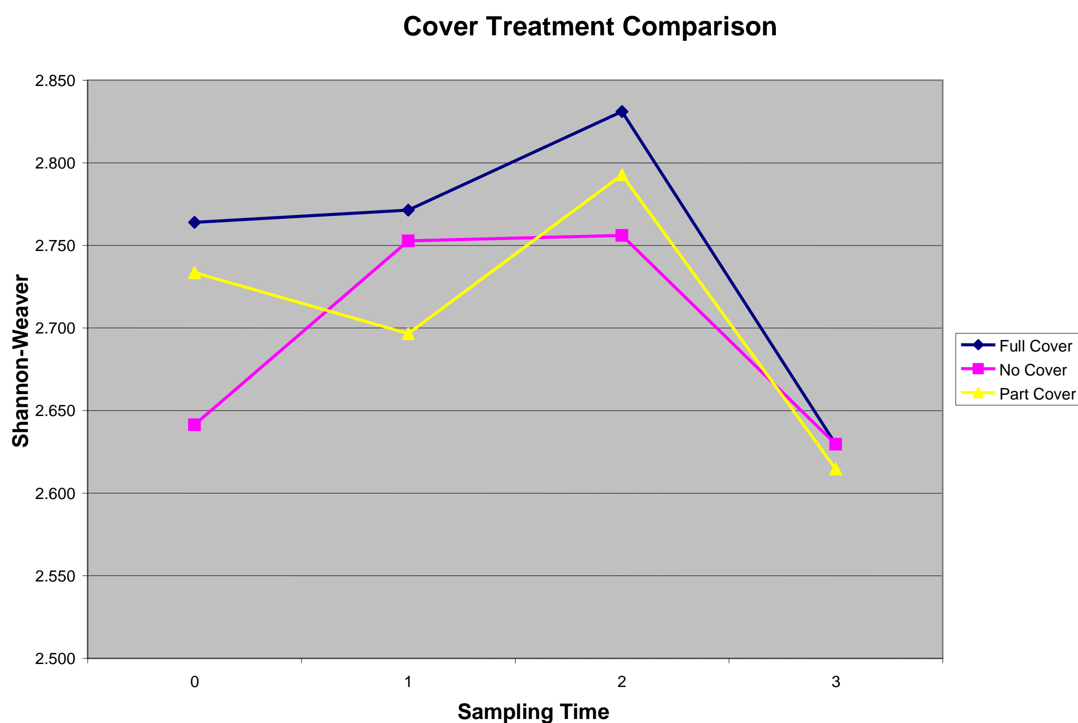


Fig. 18. Shannon-Weaver substrate diversity index values obtained for cover treatments.

Microbial diversity in all full-cover treatments except S, followed the same trend over the sampling times (Fig. 19). The upward trend of S towards the end of the season might be ascribed to the enhancement of soil bacterial populations. Although OB is marketed to have the same effect on fungal and bacterial populations as S, our analyses were unable to discern a positive effect. While ECO was one of the treatments that improved grain yields, no positive correlation could be made between grain yield and soil microbial biodiversity.

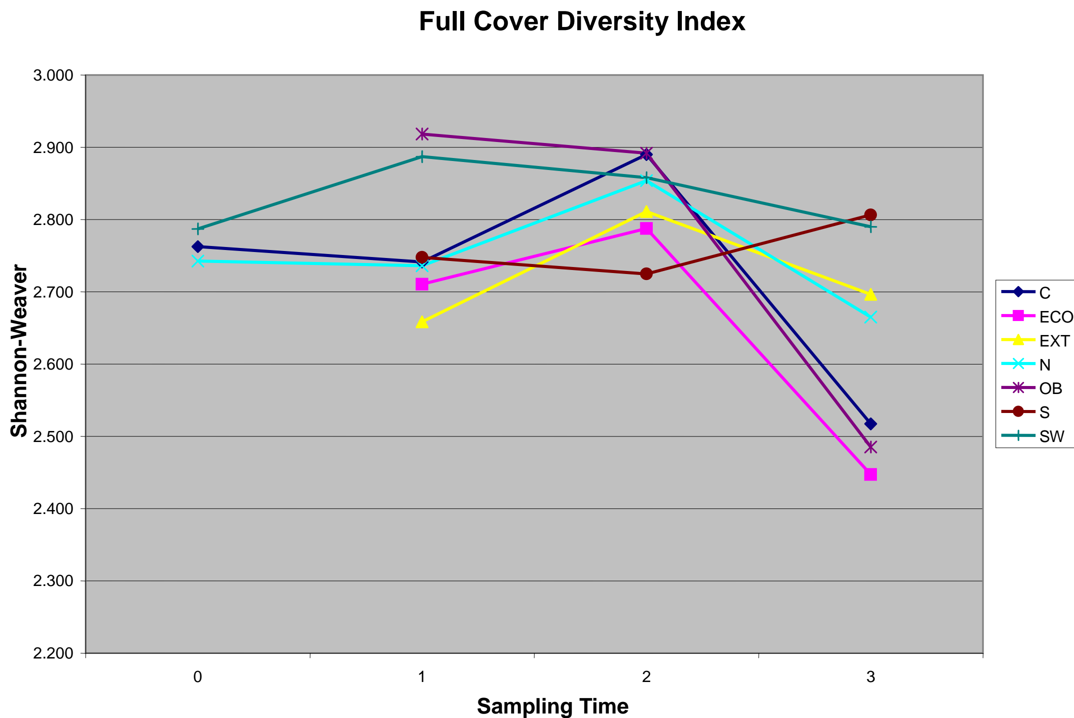


Fig. 19. Shannon-Weaver substrate diversity index values obtained for full-cover treatments.

The trend indicates an increase in microbial diversity during the second sampling time, but a sharp decline during the third sampling time. This increase in diversity could be the result of the selection of microbial communities with the ability to utilise different carbon sources present in root exudates. According to Garland (1996), the amount and composition of carbon loss from roots, or rhizodeposition, change with the age of the plant, resulting in changes in rhizosphere microbial community structure (Fig. 19).

Microbial diversity in all partial-cover treatments followed the same trend over the sampling times, except CAN and AN (Fig. 20).

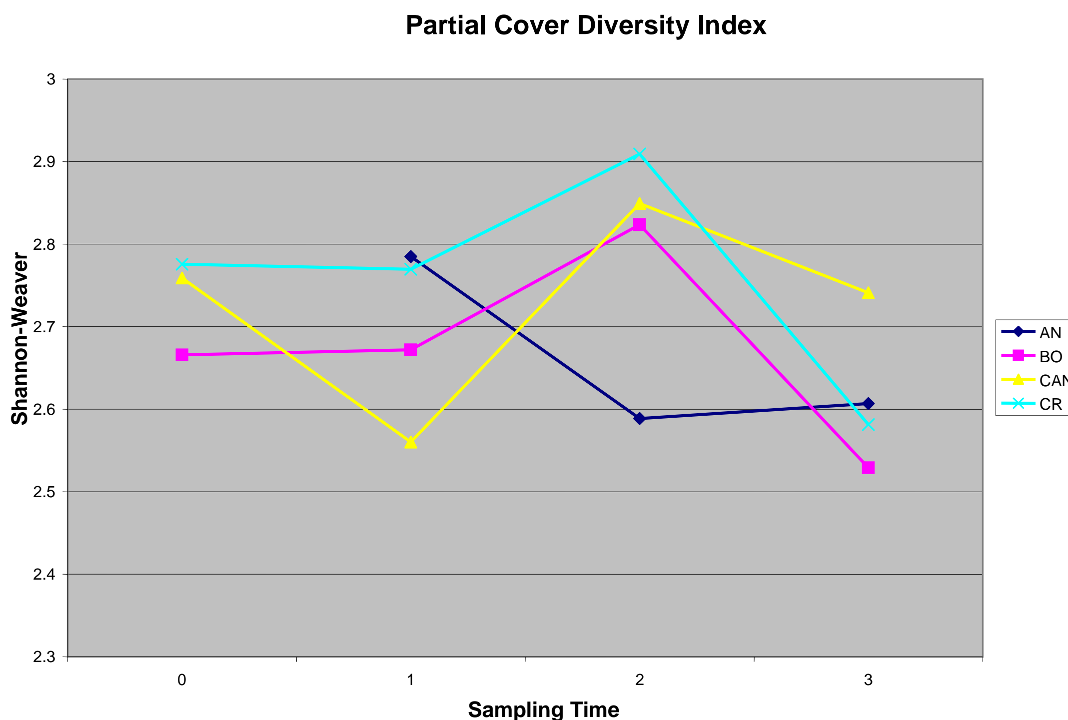


Fig. 20. Shannon-Weaver substrate diversity index values obtained for partial-cover treatments.

Microbial diversity in AN decreased between the first and second sampling, after which it stabilised. This could be attributed to the lethal levels of HNO_2 which had been created through the accumulation of NH_4-N (Tenuta & Lazarovits, 2002). The effect of AN on the biodiversity was still expressed well into the season as mentioned previously. Although AN did not return to its initial level of diversity, neither did BO or CR, and this cannot be considered a negative attribute of AN use. Despite fluctuation in CAN soil microbial diversity during the season, the index returned to its initial value. Neither the depressing effect of BO on early maize growth, nor the season-long effect of N on maize had an effect on the soil microbial biodiversity or enzyme activity.

In contrast to results obtained during the previous season (Lamprecht *et al*., 2006), microbial diversity in the MB treatment slightly increased over time. Microbial diversity fluctuations in no-cover treatments were observed, with MF fluctuating the least. SF and T had a slightly lower microbial diversity during the third sampling time, than during the first sampling time (Fig. 21). Initial and final diversity indices in SF were comparable, which is typical to a "stable" soil microbial population.

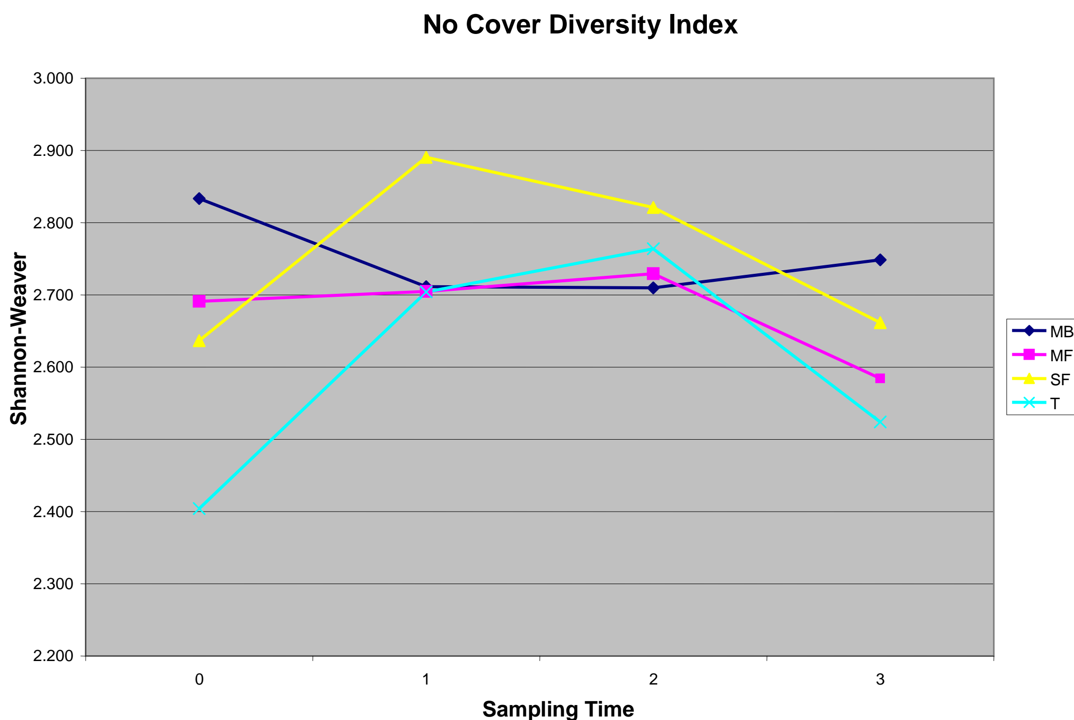
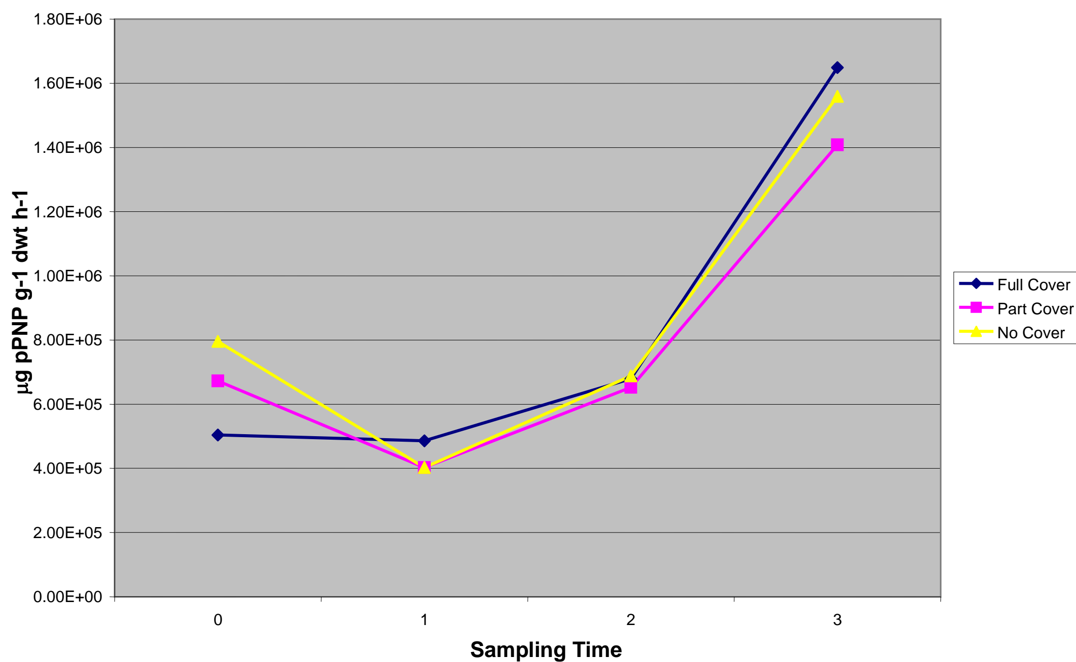


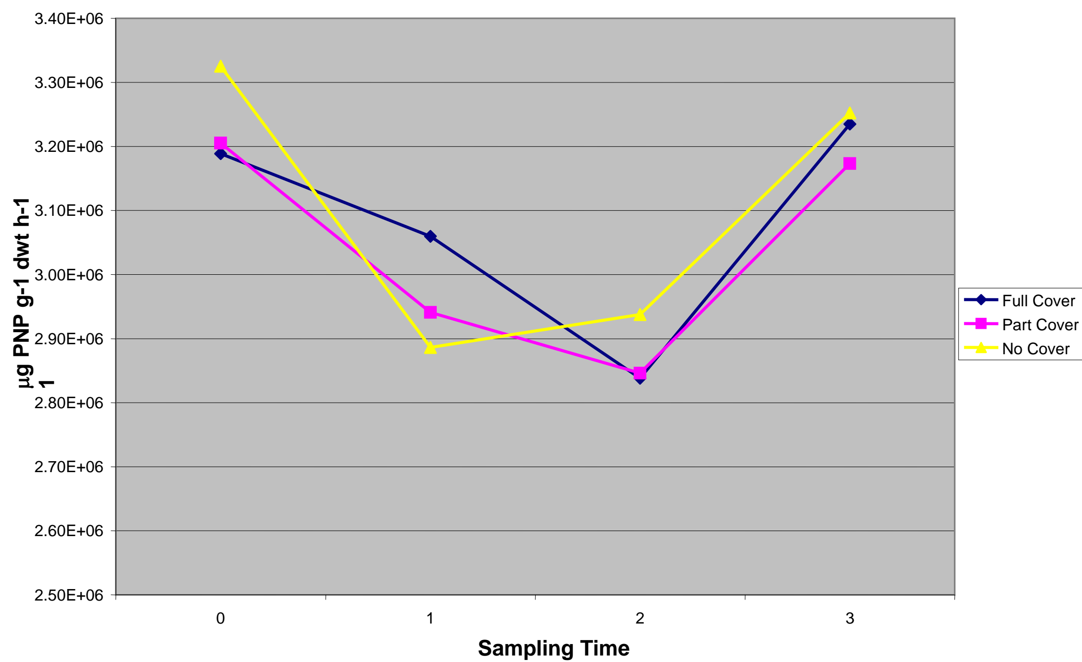
Fig. 21. Shannon-Weaver substrate diversity index values obtained for no-cover treatments.

Enzymatic activities. According to Garcia et al. (2002), enzymatic activity in the soil environment is a major contributing factor to soil microbial activity in general. ß-glucosidase (Figure 22a), acid phosphatase (Figure 22b), and urease (Figure 22c) activities were assayed because of their vital role in soil microbial activity and organic C, N and P mineralization (Dick, 1997; Deng & Tabatabai, 1997).

(a)



(b)



(c)

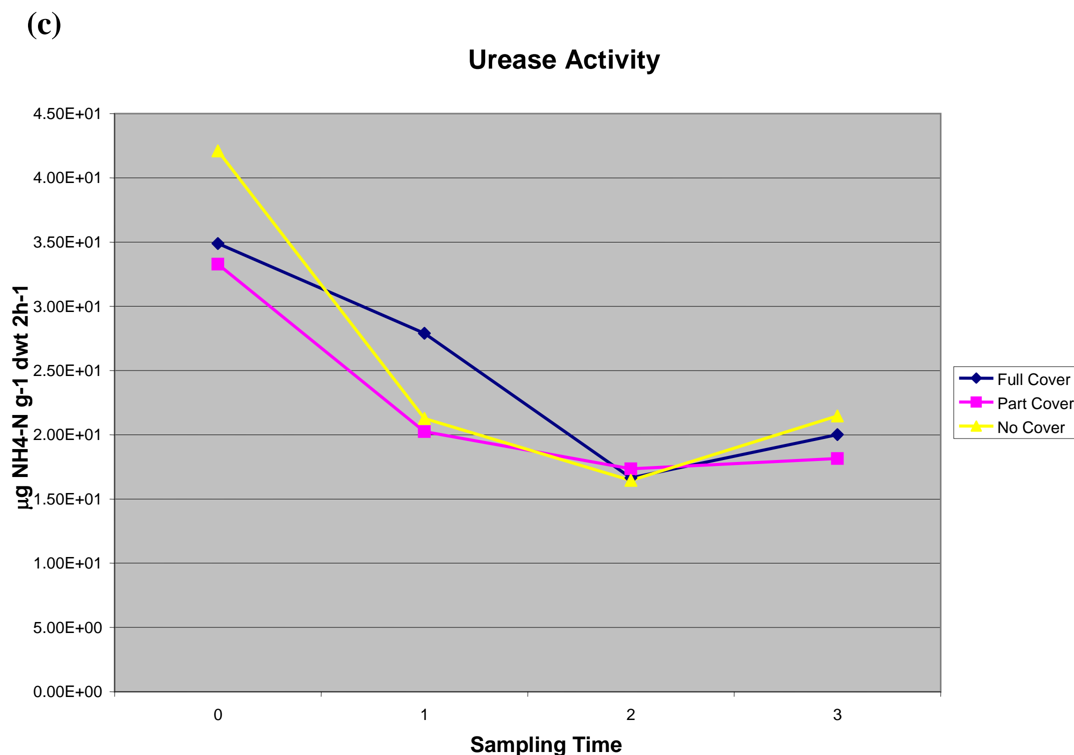


Fig. 22. **ß-glucosidase (a), acid phosphatase (b), and urease (c) activity for cover treatments.**

A gradual increase in ß-glucosidase activity could be observed over time (Figure 22a) due to stimulation of microbial activity (Martens *et al.*, 1992). The increase in β-glucosidase activity has also been useful in monitoring soil quality due to the central role it fulfils in the cycling of organic matter (Turner *et al.*, 2002).

Contrary to results obtained during the previous season (Lamprecht *et al.*, 2006), acid phosphatase activity (Fig. 23) drastically decreased until the second sampling, after which it returned to its initial levels.

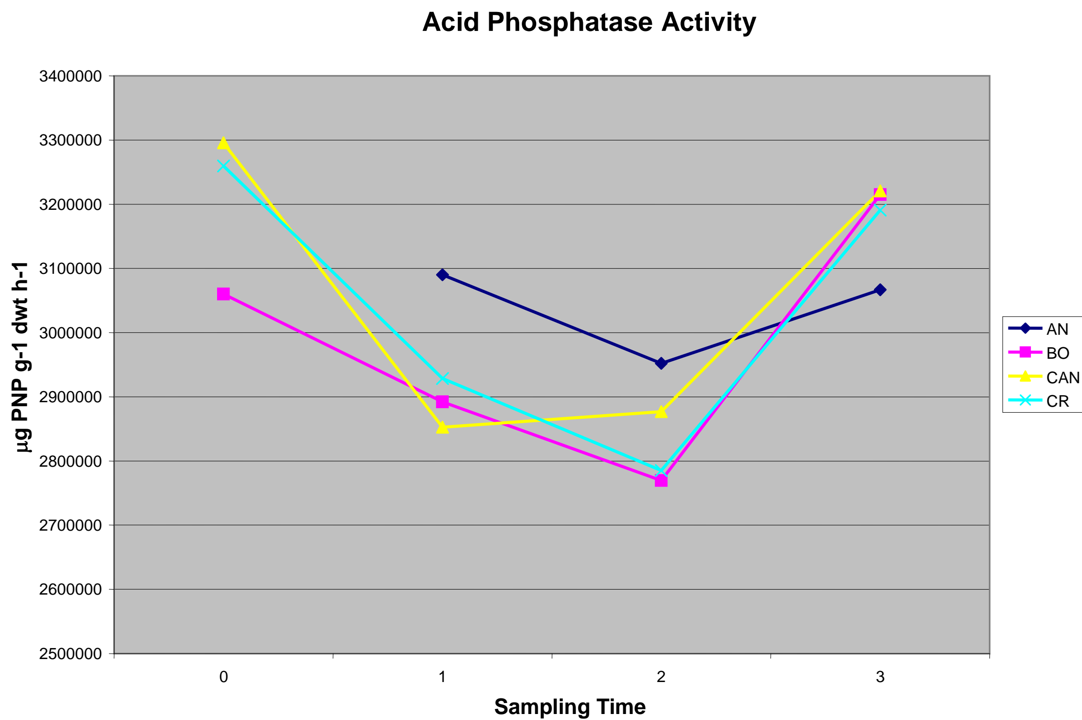


Fig. 23. Acid phosphatase activity for partial-cover treatments.

Within the partial-cover group, the AN treatment had slightly higher acid phosphatase activity (Fig. 23). It is interesting to note that the final acid phosphatase activities of the rotation crops (BO, CAN, CR) were similar. Negative correlations were found between acid phosphatase activity and both crown and root rot incidences during the second sampling.

Since urease belongs to a group of enzymes acting on C-N bonds of urea (Dick, 1997), available N decreased due to nitrogen uptake by developing maize plants, which resulted in lower urease activity (Figure 22c). SW treatments had higher urease activity during the first sampling due to the presence of nitrogen released by soybeans during the previous summer. Urease activity drastically decreased thereafter, as the available nitrogen was metabolised by the maturing maize plants.