**FINAL REPORT**

# THE ROLE AND IMPORTANCE OF SOILBORNE DISEASES IN YIELD DEPRESSION OF MAIZE

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# REPORT ON PROJECT ENTITLED "THE ROLE AND IMPORTANCE OF SOILBORNE DISEASES IN YIELD DEPRESSION OF MAIZE"

**SC Lamprecht[1], MPW Farina[3], JH Habig[2], GR Thibaud[4], and JF Bloem[2]**

[1]ARC-Plant Protection Research Institute, Private Bag X5017, Stellenbosch

[2]ARC-Plant Protection Research Institute, Private Bag X134, Pretoria

[3]27 Drew Avenue, Howick 3290

[4]KZN Department of Agriculture and Environmental Affairs, Cedara College, Private Bag X 9059, Pietermaritzburg

## EXECUTIVE SUMMARY

A field trial was conducted in KwaZulu-Natal (KZN) to identify the soilborne pathogens associated with maize following winter wheat in no-till systems and to quantify the effects on growth and grain yield. The trial was conducted at two sites (Bergville and Winterton), and fumigation with methyl bromide (MB) was used as an experimental tool to eliminate soilborne pathogens. The effects of two biocides (Eco-T and Extrasol currently being marketed to farmers) on growth and yield were assessed, as well as the effects of soil fumigation and the biocides on microbial activity and diversity. The maize cultivar PHI 32D96B was planted at both locations. The herbicide programme and aerial spray regime used to control leaf diseases was common at both sites, but herbicide toxicity was encountered in the MB-treated plots at Bergville. Soil samples were collected before planting and again when plant crowns and roots were sampled for isolations and disease severity ratings to determine microbial diversity and activity. Plant samples were collected three times with one-month intervals starting 6 weeks after planting. At the three sampling times, above ground plant parts were weighed to assess growth differences during the growing season and grain yield was determined at the end

of the season. Soil and plant chemical analyses were conducted in order to monitor nutritional effects. Fumigation of soil increased the growth of plants at both sites. This was particularly evident at Winterton during the first and second sampling, when the growth of plants in the MB plots was significantly better than the control, Eco-T – and Extrasol- treated plots. Grain yield was also significantly higher in the MB-treated plots at Winterton (16 020 kg $ha^{-1}$) than the other treatments at Winterton and Bergville. Similarly, MB caused a significant reduction in root and crown rot severity compared to the other treatments (control, Eco-T and Extrasol) at both localities. The control, Eco-T and Extrasol treatments did not differ significantly with regard to crown and root rot severity. Crown and root rot severity increased significantly at both sites from the first to the third sampling. Chemical analyses of soil and leaves showed that the application of MB had no influence on plant nutrition and that growth and yield responses were the result of improved root health. Fungi most frequently isolated from diseased crown and root tissue were *Acremonium* spp., *Fusarium equiseti*, *F. graminearum*, *F. nygamai*, *F. oxysporum*, *F. proliferatum*, *F. solani*, *F. subglutinans*, *Pyrenocheata terrestris*, *Pythium* spp. and *Trichoderma* spp. Of these, *Trichoderma* spp. followed by *F. oxysporum*, *F. graminearum* and *P. terrestris* were the most predominant. Of the fungi isolated, *F. graminearum*, *P. terrestris* and *Pythium* spp. appear to be the most important soilborne pathogens of maize according to research conducted in other countries. The incidences of the fungi were affected by sampling time, locality and treatments. Incidence of *F. graminearum* and *Pythium* spp. increased from the beginning to the end of the season, whereas *P. terrestris* was more prominent at the first and second sampling time than at the end of the season. Soil fumigation reduced the incidence of *Pythium* spp. and *P. terrestris*, but not *F. graminearum*. *Fusarium graminearum* was more frequently isolated from plants at Bergville and *Pythium* spp. and *P. terrestris* more frequently at Winterton. At harvest, it was also noted that at Winterton the MB-treated plots contained more than twice as many healthy green stalks than the other treatments, while at Bergville MB plots were no better than the others in terms of stalk rot. Carbon utilization profiles, conducted to evaluate microbial diversity in soil, separated soil treated with MB from soil subjected to the other treatments. Enzyme analyses showed higher microbial activity in the Winterton than the Bergville soil. This difference was

also reflected in the higher yields obtained at Winterton compared to Bergville. A preliminary screening of plants for the presence of plant parasitic nematodes identified a number of species and it appeared as if MB fumigation reduced the incidence of these nematodes. Although these are only single-season data, this investigation demonstrated the effects of soilborne pathogens on growth and yield reduction in maize following winter wheat in no-till systems in KZN. It also highlighted the need to establish rotational systems capable of simulating the effects of MB on plant health and to determine the relative importance of the fungi and nematodes associated with diseased maize crowns and roots. It similarly emphasized the need to determine the impact of different treatments such as crop rotation or application of biocontrol agents on microbial activity and diversity in soil and to develop markers for sustainable maize production.

## INTRODUCTION

Soilborne diseases have been shown to markedly depress dryland maize yields in continuous, conventional cropping systems (Channon & Farina, 1991). These diseases are caused by a range of pathogens of which *Fusarium* spp., *Pyrenochaeta terrestris*, *Pythium* spp. and *Rhizoctonia* spp. appear to be the most important (White, 1999). A number of fungi have been associated with diseased maize roots in South Africa, but information on the relative importance of these pathogens under local conditions is limited (Du Toit, 1968; Krüger, 1970; Scott, 1982; Deacon & Scott, 1983; Chambers 1987a,b; Smit, 1998; Smit, Van Rensburg & Rijkenberg, 1997). Although little is known about the principle soilborne pathogens of maize locally, suitable crop rotations (e.g. maize and soyabean) are recognized as having an appreciable ameliorative effect (Farina, 2000). The importance of proper crop rotations to reduce yield losses by soilborne pathogens of maize has also been reported by Williams and Schmitthenner (1963).

There is strong circumstantial evidence to indicate that soilborne diseases have an even greater detrimental effect in no-till systems (Deep & Lipps, 1996; Mannering & Griffiths, 2000; Sumner *et al*., 2002), systems currently being promoted in South Africa and internationally in the interests of soil conservation, enhancing soil biodiversity and reducing production costs (Unger, 1992). Significantly, leading no-till producing countries such as Brazil, question the viability of no-till or greatly reduced tillage systems in the absence of suitable rotations (Farina, personal communication).

The soilborne disease problem is further complicated by double cropping in irrigated agriculture. There is a limited range of crops that can be profitably produced and wheat, the most popular winter crop, is susceptible to pathogens, such as *Fusarium graminearum,* that also attack maize (White, 1999). Wheat-maize rotations consequently result in a build-up of such pathogens and detrimental effects on both crops (Schaafsma, Tamburic-Ilicic & Hooker, 2005). This has led to the frequent introduction of trash burning in an effort to reduce the problem, a practice which is not conducive to organic matter accumulation, one of the primary objectives of no-till production. Use of

soyabean as an alternative summer crop offers a partial solution, but can result in increased incidence in take-all in wheat (Huber, 1989) and, of course, maize is a crop of critical importance in this country.

Probably the weakest link in thoroughly understanding the need for rotations, and establishing an optimal cropping sequence or alternative method of intervention, is an in-depth knowledge of the disease organisms involved and a reliable measure of their effects on growth, yield and grain quality. The spectrum of soilborne diseases that dominate in no-till systems in the summer rainfall area of South Africa is unlikely to be common to those that occur in other maize producing countries, and currently there is an extreme paucity of information in this regard.

It has been reported that agricultural activities such as tillage, crop rotation and the use of pesticides and fertilizers have significant implications for microorganisms in soil (McLaughlin & Mineau, 1995; Roper & Gupta, 1995). According to Pankhurst and Lynch (1995) there is an increasing demand for information regarding the impact of management practices on the physical, chemical and biological properties of soil. This is essential to ensure the use of practices that conserve the environment and maximize profitability to farmers. While agricultural practices such as tillage, crop rotation, fertilization and irrigation are generally known to have significant effects on the physical and chemical properties of the soil, less is known of the associated changes in the biological properties (Dick, 1992). Baseline data on soil microbial populations is very limited for South African agricultural soils. Monitoring the effect of management practices on microbial diversity and activities in soil will also enable researchers to develop markers for sustainable crop production.

One such marker is the determination of functional profiles, used to measure the biological status of soil microbial population, since it relates to the actual or potential activities of organisms that contribute to ecosystem dynamics. The biogeochemical cycling of nutrients, such as carbon, nitrogen, and phosphorus is a fundamental soil function and, therefore, of great interest to assess the relative activity of soil microbial

communities (Ritz, McHugh & Harris, 2003). In this context, microbial community level physiological profiles (CLPP) and enzymatic activity assays are often analysed to determine the functional diversity of soil microbial populations. In both types of analyses, the ability of the microbial population to utilise a specific substrate is measured.

Enzyme activities, known to be influenced by soil type and soil organic matter (Dick, 1997), are early indicators of ecosystem stress and can act as biological indicators of soil degradation, compared to classical and slowly changing soil properties, such as organic matter (Dick, 1994; Garcia, Hernandez & Costa, 1994). Enzyme assays provide useful functional information on the relative presence or activity of organisms in soil microbial populations with the capacity to obtain carbon, nitrogen or phosphorous and have also been used to evaluate the fertility of the soil or to describe the functioning of the ecosystem (Aon & Colaneri, 2001).

Cropping systems that return crop residues to the field significantly increased the activity of a wide range of soil enzymes over unamended soil (Verstraete & Voets, 1977, Jordan *et al*., 1995) due to stimulation of microbial activity (Martens, Johanson & Frankenberger, 1992). Over time, crop rotation provides greater plant diversity than monoculture systems, with a positive effect on soil enzyme activities (Khan, 1970, Dick, 1984, Bolton *et al*., 1985). Stimulation of microorganisms in rhizosphere and improved physical condition of soils in crop rotations were observed particularly when rotations contained legume species (Miller & Dick, 1995a, b).

The primary aim of this study was to identify the dominant soilborne pathogens, which exist in maize following winter wheat in no-till systems and to quantify the effects on growth and final yield. Secondary objectives were to test the effects of two biocides, Eco-T and Extrasol, currently being marketed to farmers in KwaZulu-Natal (KZN), to assess the effects of soilborne diseases on nutrient uptake, and to assess soil fumigation and biocide effects on microbial diversity.

## MATERIALS AND METHODS

**Experimental site and crop management.** The trial was conducted at two localities in the Winterton / Bergville area. The soil near Winterton is a Hutton sandy clay, while that in the Bergville area is an Avalon sandy clay loam. These soil types and close variants thereof constitute the dominant cropping soils in KZN and also occur extensively in other areas of the country. The sites are under irrigation and have been in no-till production for 10 and 20 years, respectively, at Bergville and Winterton. They were cropped to wheat in the winter of 2005 and to maize the previous season. Maize stubble was burnt at Bergville, but not at Winterton. The same maize cultivar (PHI 32D96B) was used at both locations in order to eliminate the possible effects of differential cultivar susceptibility to soilborne diseases (Channon & Farina, 1991), the herbicide programme was common to both sites and so, too, was the aerial spray regime used to control leaf diseases. There were 80 m of row per plot at each site, but plot sizes were 72.8 $m^2$ at Winterton (0.91-m rows) and 60 $m^2$ at Bergville (0.75-m rows). Net plots used for sampling and final yield measurement were restricted to the inner six rows of each plot with 1 m being excluded from the end of each row. To limit across plot contamination, 1-m transverse pathways were established between plots. Both sites received adequate applications of P and K, and the total N application was 180 kg $ha^{-1}$ and 210 kg $ha^{-1}$ at Winterton and Bergville, respectively. Planting at Winterton took place on 30/11/05, a week before that at Bergville, and final stands of about 60 000 plants per ha were targeted at both localities.

**Treatments.** The treatments compared with control (normal practice) plots included methyl bromide (MB) fumigation (Fig. 1), the *Trichoderma* biocide, Eco-T, and Extrasol, a broad-based biocide which contains three *Bacillus* spp., an *Azomonas* and a *Pseudomonas* sp. MB fumigation, while not economically viable for soilborne disease control in crops such as maize, was used to provide a base-line treatment in which soilborne diseases were essentially eliminated. The two biocides involved are claimed to markedly suppress root diseases, but field evidence to support this is not readily available.

**Fig. 1. Methyl bromide application at Winterton.**

MB plots were rotovated prior to fumigation in order to ensure adequate soil penetration of the gas, which is absorbed by organic residues, and data from an adjacent existing comparison of no-till and conventional tillage at Winterton was used to estimate the effects of tillage *per se*. MB was applied at a rate of 8 kg plot$^{-1}$(1018 kg ha$^{-1}$ at Winterton and 1334 kg ha$^{-1}$ at Bergville) three weeks prior to planting. Eco-T was applied in a 50-mm wide band over the emerging seedlings in 16 L of water per plot at a rate of 0.076 g m$^{-1}$ of row. Extrasol was similarly applied at a rate of 0.125 mL m$^{-1}$ of row. The sites were then irrigated to further ensure soil penetration of the biocides.

**Chemical analysis of soil and plant material.** To monitor possible treatment effects on soil properties, topsoil samples (0-0.15 m) were collected from all plots three weeks after planting and analysed for P, K, Ca, Mg, exchangeable acidity, pH (KCl), Zn, Mn and Cu using the procedures employed routinely at Cedara College (Farina & Channon, 1988). In addition, estimates of organic carbon and clay content were obtained using near-infrared reflectance.

To measure possible treatment and locality effects on nutrient recovery by the crop, whole plant samples collected during the first sampling and leaf samples (leaf opposite to and below the primary ear) collected at silking were analysed for N, P, K, Ca, Mg, Zn, Mn, Cu, and B by standard procedures.

**Plant sampling for isolations and disease severity ratings.** Plant samples were collected on 9/1/06, 6/2/06 and 8/3/06 (Fig. 2). Ten pants per plot were sampled during the first and third sampling, but time constraints resulted in only five plants per plot being removed at the second sampling. All plants collected were rated for root and crown rot on a scale where 0 represented healthy crowns and roots, 1 = >0 – 25% rot, 2 = >25 – 50% rot, 3 = > 50 – 75% rot and 4 = > 75 – 100% rot.

**Fig. 2. Washing roots and weighing above ground plant parts at Bergville.**

**Isolation from plant material.** Isolations were done from all plants collected during the first, second and third sampling. Plant roots were washed under running tap water to remove adhering soil, surface disinfested in 1% sodium hypochlorite, rinsed twice in sterile distilled water, and allowed to dry in a laminar flow cabinet after rinsing. Small

pieces of diseased root and crown tissue were excised and plated onto each of the following growth media: water agar (WA), water agar with 0,02 % novostreptomycin (WA+), potato dextrose agar with 0,02 % novostreptomycin (PDA+), selective *Fusarium* agar (SFA) and *Pythium* selective medium (PARP). Forty pieces of plant material (twenty root and twenty crown) were plated per plot. All fungi that developed were transferred to divided Petri dishes containing carnation leaf agar (WA with sterile carnation leaves) in one half and PDA+ in the other. Cultures were incubated at 20 – 22°C under near-ultraviolet light with a 12-h photoperiod. All fungi were identified and recorded.

**Estimation of microbial activity.**

*Soil samples*

Soil samples were collected aseptically at four sampling stages during the season to determine the functional diversity of microbial populations in soil. Three composite samples were randomly taken from each plot to a depth of ±20cm, and samples stored at ±5°C prior to analyses. Sub-samples for enzyme activities were dried at 40°C for 48h, sieved (<2mm) and stored at ±5°C.

***Determination of functional diversity***

Whole-community substrate utilisation patterns are assessed when a carbon source is utilised. Soil dilutions (1:3,000) (Buyer & Drinkwater, 1997) were prepared to allow for the recovery of several types of bacteria, and to retain numerically abundant organisms while eliminating fast-growing competitors (De Fede, Panaccione & Sexstone, 2001). The soil suspensions were aseptically inoculated into the EcoPlates™ and incubated at 25°C. Respiration of carbon sources by microbial populations reduced the tetrazolium dye, causing a colour change. This colour change was spectrophotometrically quantified twice daily over a period of 10 days at a wavelength of 590nm (Winding & Hendriksen, 1997). The 48h-data were used to calculate the average well colour development (Garland & Mills, 1991).

***Determination of soil enzyme activity***

*β-glucosidase activity (C)*

The ability of a soil population to obtain carbon was determined according to Dick, Breakwell and Turco. (1996). The *p*-nitrophenol released after the incubation of soil with *p*-nitrophenyl glucoside (PNG) solution was measured. A calibration curve was prepared and used to calculate the *p*-nitrophenol $mL^{-1}$ of the filtrate per soil sample.

*Phosphatase - phosphomonoesterase activity (P)*

The ability of a soil population to obtain phosphorous was determined according to Dick *et al.,* (1996). The *p*-nitrophenol released after the incubation of soil with *p*-nitrophenyl phosphate (PNP) solution was measured. A calibration curve was prepared and used to calculate the *p*-nitrophenol $mL^{-1}$ of the filtrate per soil sample.

*Urease (N)*

The ability of a soil population to obtain nitrogen was determined using the method of Kandeler and Gerber (1988). Released ammonia is measured after the incubation of soil samples with a urea solution. The results were calculated with reference to the calibration curve.

**Growth and yield measurements.** When collecting root samples, above ground plant parts were weighed in order to assess growth differences during the growing season Fig. 1). At harvest, all plants remaining in the plots were counted so as to facilitate yield determinations on a per plant basis. Grain yield per plot was then expressed on a per ha basis using the original mean stand and corrected to a moisture content of 12.5 %.

**Statistical analysis.** The experimental design was a complete block experiment with four treatments replicated in three blocks at two localities. The localities were regarded as the main plot factor and treatments as a sub-plot factor. A standard split-plot ANOVA was performed for the dry mass, wet weight, chemical composition of soil and

plant material data. For the crown and root rot ratings and the incidences of fungi data, the repeated measurements over three times were added as a further sub-plot factor to the ANOVA. Student's t-least significant differences at the 5% level were calculated to compare the effect of localities, sampling times and treatments on the parameters that were measured.

Data on carbon utilization were subjected to non-parametric statistical analyses using STATISTICA 6 (StatSoft, Inc ©). Carbon substrate utilisation profiles were statistically analysed by principal component analysis (PCA) (Palojärvi *et al.*, 1997). Biodiversity was determined using the Shannon-Weaver diversity index, which indicates species richness and the proportion of each species within the local soil microbial community (Magurran, 1988).

## RESULTS AND DISCUSSION

**Growth, final yield, and leaf and soil analysis.** At Winterton, emergence and early growth were considerably better in MB-treated plots (Fig. 3). Similar effects were initially evident at Bergville, but subsequent to a post-emergence application of metolachlor, plants in fumigated plots displayed severe symptoms of metolachlor toxicity (Fig. 4).

**Fig. 3. Methyl bromide plots at Winterton (right of the white peg, left back and right back) versus a control plot (left of the white peg).**

This effect was evident for some two to three weeks and is likely to have compromised the MB treatment at this site. Reasons for this negative effect are not immediately clear, but possibly resulted from an over application of metolachlor on rotovated plots, which was associated with an absence of surface stubble residue (less absorption of the chemical) and/or an excessively high application rate. It is noteworthy, that although the clay content of the Bergville site was approximately half that of the soil at Winterton, the

rate of metolachlor applied was the same. Other possible contributory factors include the wetter conditions that existed at Bergville, the shallower and poorly drained nature of the soil and the fact that the MB application rate per ha was higher. MB may have delayed the breakdown of metolachlor.

**Fig. 4. Metolachlor damage in a methyl bromide plot at Bergville.**

Another feature of the MB-treated plots at both sites was the considerably greater number of suckers that developed prior to herbicide application, and earlier flowering. The latter effect was particularly marked at Winterton (Fig. 5).

**Fig. 5. A methyl bromide plot at Winterton already flowering and considerably taller than the adjacent treatments.**

Treatment effects on growth and final grain yield are shown in Table 1. At the first sampling time only the MB treatment at Winterton was significantly superior This was also the case at the second sampling time and at harvest, but at the third sampling, while the same trend was evident, this treatment was not statistically superior to other treatments at Winterton or to the MB treatment at Bergville. It is also noteworthy, that from the second sampling time the overall superiority of the Winterton plots tended to increase and that it was appreciable in terms of final grain yield. The negative effect of metolachlor at Bergville may have been a contributory factor, but it is also likely that general growing conditions and a severe late-season infection of Grey Leaf Spot (*Cercospora zeae-maydis)* played a role. At harvest, the consequent high incidence of stalk rots at this site was clearly evident, while at Winterton, in spite of the trial having been earlier planted, there were green leaves still evident in several plots. In this regard, it is noteworthy that at Winterton the MB-treated plots contained more than twice as many healthy, green stalks than the other treatments, while at Bergville MB plots were no better than the others in terms of stalk rot and there was a total absence of green leaf. There had clearly been some curtailment of vegetative growth at Bergville.

**Table 1. Treatment effect on plant mass at three sampling times and on grain yield.**

| Location | Treatment | Sampling Time[a] | | | Grain Yield |
|---|---|---|---|---|---|
| | | 1[b] | 2[c] | 3[d] | |
| | | ---------- kg plot$^{-1}$ ---------- | | | kg ha$^{-1}$ |
| Bergville | Control | 0.169 b | 4.880 cd | 11.640 c | 12270 ed |
| | Eco-T | 0.167 b | 4.660 d | 12.827 bc | 12180 e |
| | Extrasol | 0.162 b | 4.627 d | 12.567 bc | 13230 cde |
| | Methyl bromide | 0.212 b | 5.517 bc | 13.353 abc | 13000 cde |
| Winterton | Control | 0.176 b | 5.660 bc | 14.017 ab | 14500 b |
| | Eco-T | 0.164 b | 5.720 b | 13.547 ab | 13470 bcd |
| | Extrasol | 0.196 b | 5.133 bcd | 14.053 ab | 14050 bc |
| | Methyl bromide | 0.375 a | 6.730 a | 15.053 a | 16020 a |

[a]Means within a column followed by the same letter do not differ significantly (P=0.05)
[b]Oven dry mass of 10 plants
[c]Wet mass of five plants
[d]Wet mass of 10 plants

A final important feature of the growth data provided in Table 1 is the fact that at no stage was there a significant benefit due to either Eco-T or Extrasol.

The superiority of the MB treatment at Winterton in terms of final grain yield was striking, particularly in the light of the good season that was experienced and the exceptional yields obtained. Reasons for this superiority constitute the core of this investigation and before examining treatment effects on soilborne disease incidence, it is necessary to examine the role of other possible contributory factors, such as the effect of tillage *per se* in MB-treated plots and the role of fumigation on nutrition (Channon & Farina, 1991).

Two-year data from an adjacent trial at Winterton in which continuous no-till is being compared with annual ploughing and annually alternated no-till and conventional tillage indicate that, in the presence of adequate N, no tillage effect is measurable (G. Thibaud, unpublished data). This suggests that any benefit from tillage in the MB-treated plots can probably be discounted.

The role of treatment on nutrition, assessed from whole plant (first sampling time) and leaf analytical data, indicated that there were no meaningful treatment effects (data not shown) and that the levels of all nutrients measured were more than adequate. There were, however, significant differences between sites. Whole plant samples from the Bergville site were significantly higher in S (0.25 % vs 0.22 %) and plants from Winterton contained significantly more P (0.47 % vs 0.45 %), K (6.09 % vs 5.72 %), Ca (0.44 % vs 0.39 %), Zn (79 mg $kg^{-1}$ vs 56 mg $kg^{-1}$) and Mn (67 mg $kg^{-1}$ vs 56 mg $kg^{-1}$). Available soil S was not determined, but differences noted in the plant content of P, K, Ca, Zn and Mn were consistent with differences in the topsoil content of these elements (Table 2 ).

**Table 2. Significantly different physical and chemical properties of the topsoil (0-15 cm) at Bergville and Winterton three weeks after planting.**

| Location | P | K | Ca | Mg | Zn | Mn | Cu | Clay | Org. C |
|---|---|---|---|---|---|---|---|---|---|
| | ----------mg $L^{-1}$ ---------- | | | | | | | -------%------- | |
| Bergville | 52 | 333 | 868 | 206 | 7.7 | 14 | 2.8 | 24 | 1.7 |
| Winterton | 98 | 381 | 1017 | 173 | 17 | 24 | 3.7 | 45 | 2.0 |

Leaf samples collected at flowering similarly showed that no significant treatment effects had occurred (data not shown). Most across-site differences evident in the earlier sampling had disappeared and only the strong Mn advantage at Winterton was still evident (84 mg $kg^{-1}$ vs 66 mg $kg^{-1}$). In addition, however, at this sampling stage plants at the Bergville site contained significantly more Mg (0.23 % vs 0.19 %), on average. This is also consistent with differences evident in terms of soil analysis (Table 2). At this sampling stage, too, the nutrient levels were more than adequate and it is considered most unlikely that mineral nutrition played any role in the yield effects shown in Table 1.

Topsoil analyses indicated that, while there were significant differences between sites (Table 2), these were, with the notable exception of Ca and Mg, not influenced by treatment. In the case of Ca and Mg, both elements were significantly lower in the MB plots. However, this effect, not evident in terms of plant analysis, was almost certainly brought about by the dilution effect introduced by tillage prior to applying MB. In no-till systems, surface liming is employed to reduce anthropogenic acidification induced by N

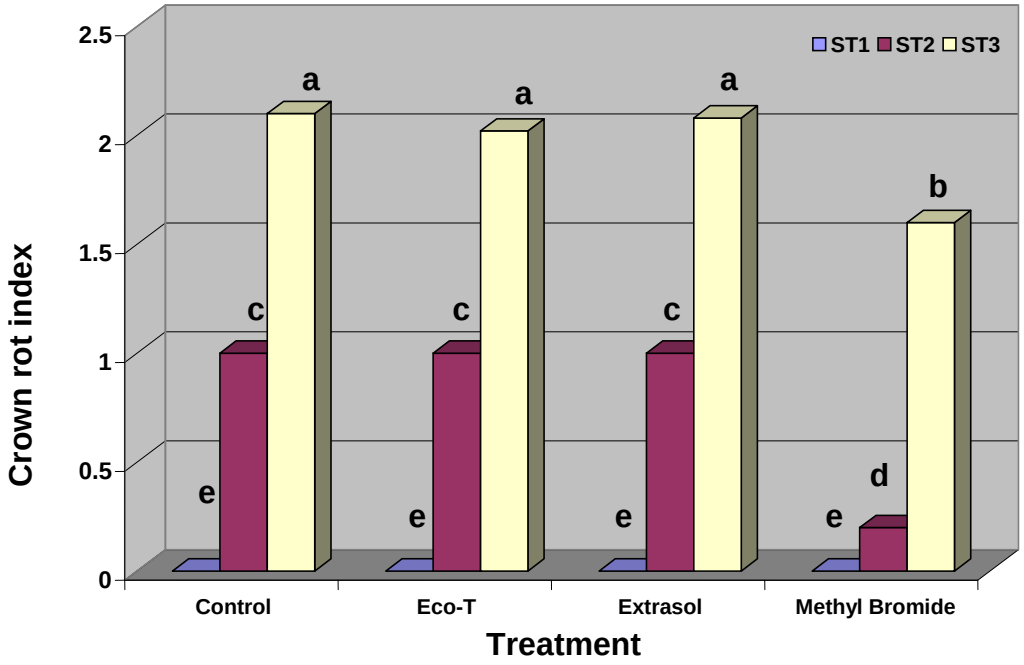
topdressings (Thibaud, 2000). This results in a marked surface build-up of Ca and Mg and rotovation would have diluted these elements into the immediate subsoil.

In summary, then, it appears that tillage and the treatments imposed had no influence on plant nutrition. This is at variance with earlier work done in conventional, rainfed systems (Channon & Farina, 1991), but in view of the exceptionally high levels of nutrition at the two sites (Table 2), is not surprising. Although the trials were irrigated and the climatic conditions experienced were above average, it appears that the effects of MB must have resulted from the superior moisture recovery that would be anticipated from improved root health, something that would have been more relevant in the freely drained Hutton soil at Winterton than in the shallower and generally wetter Avalon soil at Bergville.

**Crown and root rot severity.** Roots from MB-treated plots were markedly less diseased than roots from the other treatments at all the sampling times. Differences evident at the second sampling time are illustrated in Figures 6a, b, c and d. Crown and root rot severity were significantly affected by treatment and significant treatment × sampling time interactions (crowns = $P < 0.0001$; roots = $P = 0.0033$) occurred. Crowns from MB-treated plots were significantly ($P \leq 0.05$) less diseased than crowns from the control and other treatments at the second and third sampling times and roots significantly less diseased than roots from the other treatments at all sampling times (Fig. 7a, b). Crown and root rot severity of plants from the control, Eco-T and Extrasol treated plots did not differ significantly (Fig. 7a, b). Fumigation of soil as an experimental tool to reduce soilborne diseases has been successfully used by researchers to study the effects of soilborne diseases on yield in maize and wheat. Channon and Farina (1991) in South Africa and Sumner *et al*., (1990) in the USA showed that fumigation with BUSAN 1020 (sodium N-methyldithiocarbamate) and DD-MENCS (20% methyl isothiocyanate + 80% chlorinated C3 hydrocarbons), respectively, reduced root diseases of maize. Similarly, Cook, Sitton and Haglund (1987), Scott, Kilian and Miles, (1992) and Crafford *et al*., (unpublished) reported that the increased yield responses of wheat following soil fumigation could be attributed to the control of soilborne diseases.


**Fig. 6. Roots at the second sampling (Control a, Methyl bromide b, Extrasol C, and Eco-T d).**

(a)




(b)

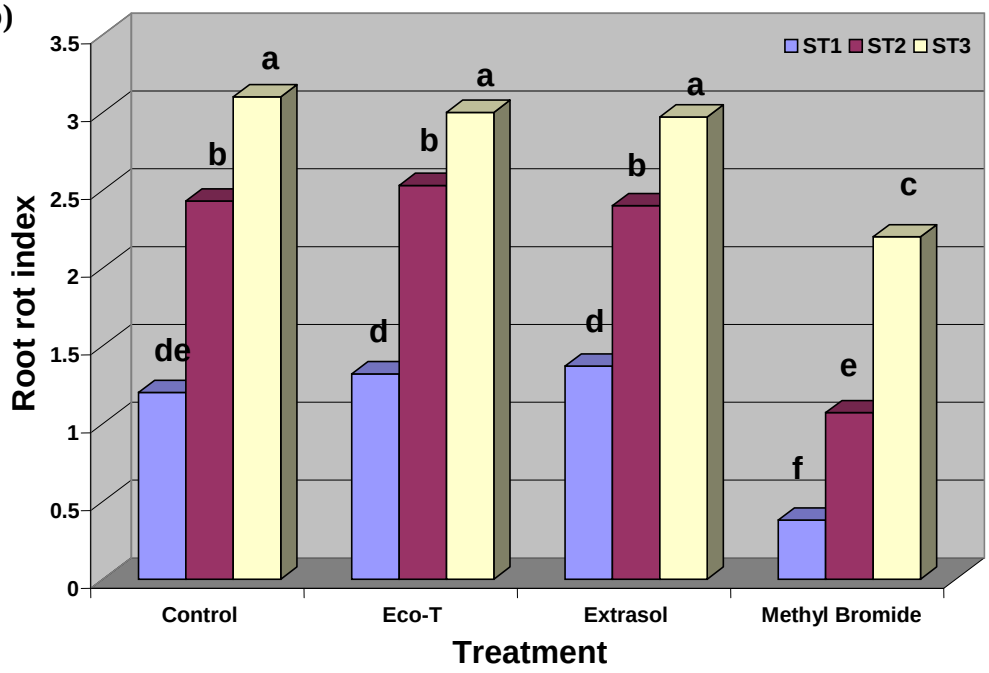



**Fig. 7. Effect of treatment and sampling time (ST) on crown and root rot severity of plants.**

No crown rot was detected during the first sampling time, but both crown and root rot severity significantly increased ($P \leq 0.05$) from the first to the second sampling at both localities (Fig. 8a, b). A significant ($P = 0.0038$) locality × sampling time interaction was recorded for root rot severity.

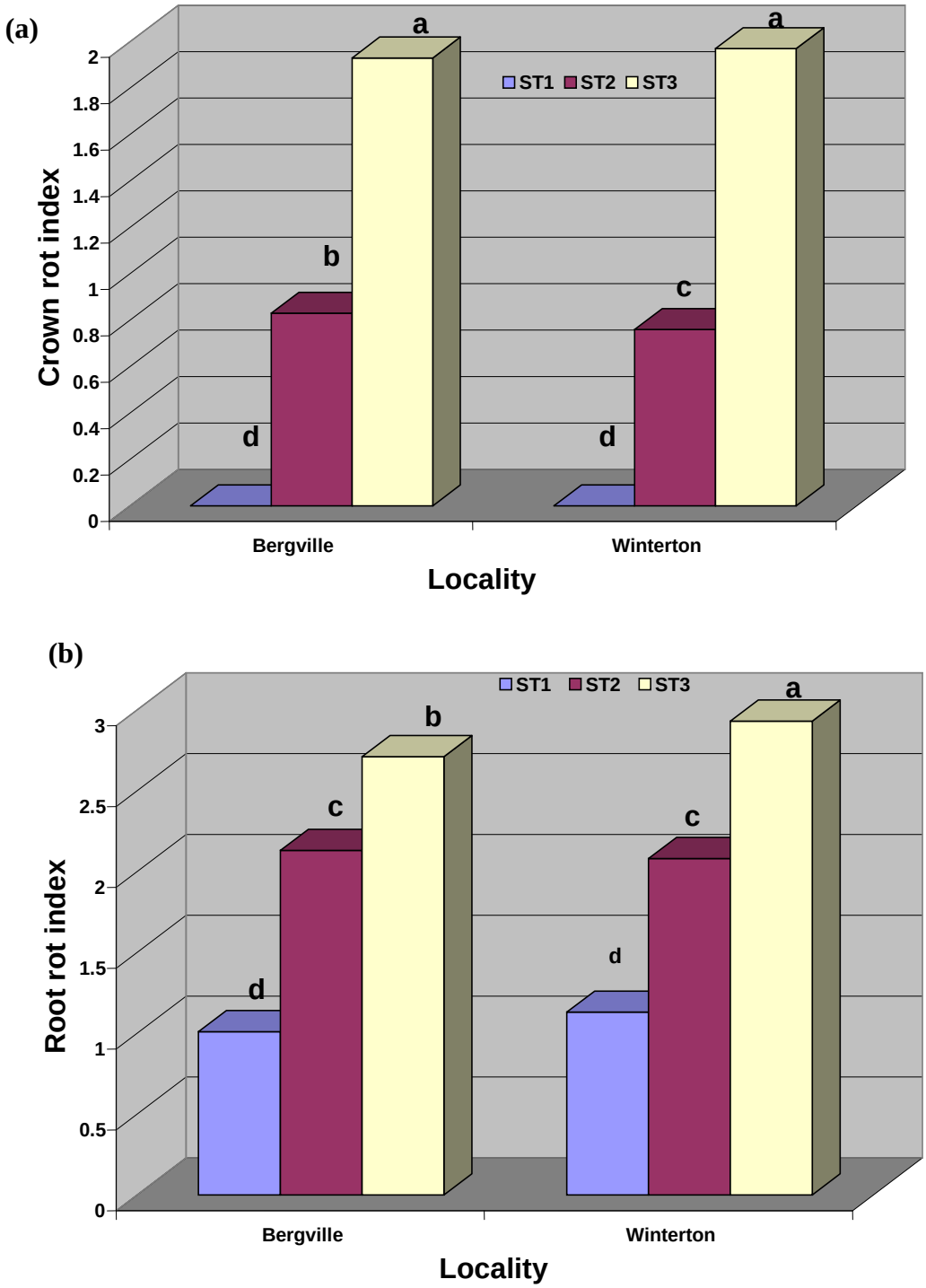



**Fig. 8. Effect of locality and sampling time (ST) on crown and root rot severity of plants.**

**Incidence of fungi in roots and crowns.** A number of fungi were isolated from crowns and roots in this study. Fungi infrequently isolated are listed in Table 3 and were not subjected to statistical analyses. Fungi most frequently isolated from crowns and roots were *Acremonium* spp., *Fusarium equiseti*, *F. graminearum*, *F. nygamai*, *F. oxysporum*, *F. proliferatum*, *F. solani*, *F. subglutinans*, *Pyrenocheata terrestris*, *Pythium* spp. and *Trichoderma* spp. Sampling time, location and treatment time affected the incidences of these fungi in the crowns and roots (Table 4). All fungi were isolated from crowns and roots, except *Pythium* spp. that were only isolated from roots. *Acremonium* spp. and *F. proliferatum* were isolated more frequently from crowns than roots of plants. All of the fungi frequently isolated, except *F. nygamai*, have been previously recorded as soilborne pathogens of maize. Of the less frequently isolated fungi, *F. acuminatum*, *F. verticillioides* and *Rhizoctonia* spp. are known as soilborne pathogens of maize (Sumner & Bell, 1982; Ramsey, 1990a, b). 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 3. Fungi infrequently isolated from crowns and roots of maize plants collected at Bergville and Winterton.**

| Fungus | Bergville[a] | | Winterton[a] | |
|---|---|---|---|---|
| | **Crowns** | **Roots** | **Crowns** | **Roots** |
| *Alternaria* spp. | + | + | + | + |
| *Aspergillus niger* | - | - | + | - |
| *Aspergillus* spp. | + | + | + | + |
| *Auroebasidium* spp | - | - | - | + |
| *Bipolaris* spp. | - | + | + | + |
| *Chaetomium* spp. | + | + | + | + |
| *Cladosporium cladosporioides* | + | - | + | - |
| *Colletotrichum* sp. | - | - | - | + |
| *Diplodia* spp. | - | - | + | + |
| *Epicoccum* spp. | + | + | + | + |
| *Fusarium acuminatum* | - | + | - | - |
| *Fusarium pseudograminearum* | - | - | - | + |
| *Fusarium scirpi* | - | + | + | - |
| *Fusarium semitectum* | - | - | - | + |
| *Fusarium* spp. (unidentified) | + | + | + | + |
| *Fusarium reticulatum* | - | + | - | - |
| *Fusarium verticillioides* (syn. *Fusarium moniliforme*) | - | - | + | + |
| *Gliocladium catenulatum* | - | - | + | - |
| *Gliocladium roseum* | + | + | + | + |
| *Macrophomina phaseolina* | - | - | - | + |
| *Mortierella* spp. | - | + | + | - |
| *Mucor* spp. | + | + | + | + |
| *Myrothecium* spp. | + | - | - | - |
| *Paecilomyces lilacinus* | + | - | - | - |
| *Penicillium* spp. | + | + | + | + |
| *Periconia* spp. | - | + | - | + |
| *Phoma* spp. | - | + | + | + |
| *Rhizoctonia* spp. | - | + | - | + |
| *Rhizopus* spp. | + | - | + | + |
| *Stenocarpella maydis* (syn. *Diplodia maydis*) | - | - | + | + |
| Sterile fungi | + | + | + | + |

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

**Table 4. Effect of location, treatment and sampling time on the incidences of fungi in roots and crowns.**

| Factors | Plant Part | Fungi [a] | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **Acrem** | **Fequi** | **Fgram** | **Fnyga** | **Foxys** | **Fprol** | **Fsola** | **Fsubg** | **Pyren** | **Pythi** | **Trich** |
| Location (L) | Crown | NS | NS | * | NS | NS | * | NS | NS | NS | NS | NS |
| | Root | NS | NS | NS | NS | NS | * | NS | NS | * | NS | * |
| Treatment (T) | Crown | NS | NS | NS | NS | NS | * | NS | NS | NS | NS | NS |
| | Root | NS | NS | * | NS | * | NS | NS | NS | NS | * | NS |
| Sampling time (S) | Crown | * | * | * | * | * | NS | NS | NS | NS | NS | NS |
| | Root | NS | * | * | * | * | * | NS | NS | * | NS | NS |
| L x T | Crown | NS | NS | NS | NS | NS | * | NS | NS | NS | NS | * |
| | Root | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| L x S | Crown | NS | NS | * | NS | NS | NS | NS | NS | NS | NS | NS |
| | Root | NS | * | NS | NS | NS | * | NS | NS | * | NS | * |
| T x S | Crown | NS | * | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| | Root | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| L x T x S | Crown | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| | Root | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |

[a] 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.
[b] NS = not significant, * significant at P = 0.05

*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 the crowns of plants. *Trichoderma* spp. and *F. graminearum* were more prevalent on plants at Bergville and *P. terrestris* on plants at Winterton (Tables 5 and 6). There was no significant difference in the incidence of *F. oxysporum* at Bergville and Winterton.

**Table 5. Effect of location on the incidences of fungi isolated from roots and crowns.**

| Fungus | Plant Part | Incidence (%)[a] | |
|---|---|---|---|
| | | **Bergville** | **Winterton** |
| *Acremonium* sp. | Crown | 2.78a | 3.06a |
| | Root | 0.00a | 0.14a |
| *Fusarium equiseti* | Crown | 0.28a | 0.28a |
| | Root | -[b] | - |
| *Fusarium graminearum* | Crown | - | - |
| | Root | 23.06a | 17.92a |
| *Fusarium nygamai* | Crown | 1.39a | 0.69a |
| | Root | 2.64a | 3.61a |
| *Fusarium oxysporum* | Crown | 6.67a | 4.03a |
| | Root | 30.97a | 24.72a |
| *Fusarium proliferatum* | Crown | - | - |
| | Root | - | - |
| *Fusarium solani* | Crown | 0.83a | 0.14a |
| | Root | 7.50a | 4.17a |
| *Fusarium subglutinans* | Crown | 1.11a | 0.97a |
| | Root | 0.97a | 1.39a |
| *Pyrenochaeta terrestris* | Crown | 0.97a | 0.56a |
| | Root | - | - |
| *Pythium* spp. | Crown | 0.00a | 0.00a |
| | Root | 1.94a | 3.33a |
| *Trichoderma* spp. | Crown | - | - |
| | Root | - | - |

[a] Means within a fungus within a row followed by the same letter do not differ significantly ($P = 0.05$)
[b] - See Tables 6 & 9 for data on significant interactions

**Table 6. Effect of location and time on the incidence of fungi on crowns and roots of plants.**

| Fungus | Plant Part | Location | Incidence (%)[a] | | |
|---|---|---|---|---|---|
| | | | Sampling time 1 | Sampling time 2 | Sampling time 3 |
| *Fusarium equiseti* | Root | Bergville | 2.08a-c | 4.17a | 1.67bc |
| | | Winterton | 0.00c | 2.08a-c | 3.75ab |
| *Fusarium graminearum* | Crown | Bergville | 0.42b | 1.25b | 7.08a |
| | | Winterton | 0.00b | 0.42b | 0.83b |
| *Fusarium proliferatum* | Root | Bergville | 0.42b | 0.83b | 0.42b |
| | | Winterton | 2.92b | 2.92b | 9.17a |
| *Pyrenochaeta terrestris* | Root | Bergville | 3.75bc | 3.75bc | 0.42c |
| | | Winterton | 15.42a | 20.00a | 5.42b |
| *Trichoderma* spp. | Root | Bergville | 49.17ab | 36.67c | 41.67bc |
| | | Winterton | 54.58a | 32.91c | 15.42d |

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

Incidence of *Trichoderma* spp. in crowns did not differ significantly at the three sampling times, but in roots the fungi decreased significantly from the first to the second sampling time at Winterton, but not Bergville (Table 6). *Trichoderma* spp. have been listed as both pathogens of maize and biocontrol agents for maize diseases (McFadden & Sutton, 1975; Elad, Zvieli & Chet, 1986, Mao *et al*., 1997). McFadden and Sutton (1975) showed that *T. koningii*, *T. harzianum* and *T. hamatum* can produce first internode lesions in maize seedlings. According to Hornby and Ullstrup (1967) and Whitney and 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. The *Trichoderma* species obtained in this study were not identified to species level due to financial constraints and it is, therefore, not possible to determine the pathogenic potential of the *Trichoderma* populations in this study. It is also not possible to determine how the different treatments, localities and sampling times affected the *Trichoderma* spp. included in the Eco-T treatment.

The second most prominent fungus obtained in this study was *F. oxysporum*. This fungus increased significantly on crowns from the first to the third sampling time and there were no significant differences in the incidences at Bergville and Winterton (Tables 5 and 7). The 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 and Kommedahl (1969) and Warren and 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.

**Table 7. Effect of sampling time on the incidences of fungi in crowns and roots.**

| Fungus | Plant Part | Incidence (%)[a] Sampling time 1 | Sampling time 2 | Sampling time 3 |
|---|---|---|---|---|
| *Acremonium* sp. | Crown | 0.83b | 1.25b | 6.67a |
| | Root | 0.00a | 0.00a | 0.21a |
| *Fusarium equiseti* | Crown | 0.00b | 0.00b | 0.83a |
| | Root | 1.04b | 3.13a | 2.71ab |
| *Fusarium graminearum* | Crown | - | - | - |
| | Root | 8.13b | 12.92b | 41.42a |
| *Fusarium nygamai* | Crown | 2.08a | 0.12b | 0.83ab |
| | Root | 5.83a | 1.67b | 1.88b |
| *Fusarium oxysporum* | Crown | 3.33b | 4.58ab | 8.13a |
| | Root | 22.71b | 34.17a | 26.67b |
| *Fusarium proliferatum* | Crown | 3.13a | 2.92a | 4.38a |
| | Root | 1.67b | 1.88b | 4.79a |
| *Fusarium solani* | Crown | 0.00a | 0.83a | 0.63a |
| | Root | 5.83a | 3.54a | 8.13a |
| *Fusarium subglutinans* | Crown | 0.63a | 0.63a | 1.88a |
| | Root | 1.25a | 0.83a | 1.46a |
| *Pyrenochaeta terrestris* | Crown | 0.42a | 0.83a | 1.04a |
| | Root | 9.58a | 11.88a | 2.92b |
| *Pythium* spp. | Crown | 0.00a | 0.00a | 0.00a |
| | Root | 0.42b | 3.13a | 4.38a |
| *Trichoderma* spp. | Crown | 10.00a | 6.46a | 10.00a |
| | Root | 51.88a | 34.79b | 28.54b |

[a] Means within a fungus within a row followed by the same letter do not differ significantly (P = 0.05)
[b] - See Tables 6 & 9 for data on significant interactions

*Fusarium graminearum* increased significantly in roots at both localities from the first (8%) to the third sampling time (41%) and on crowns at Bergville (Tables 6 and 7). Ramsey (1990b) also reported higher incidences of *F. pseudograminearum* after physiological maturity than earlier in the season. The high incidences of *F. graminearum* in

maize crowns and roots in our study are not unexpected, since wheat was the preceding crop. 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). Furthermore, *F. graminearum* produces mycotoxins such as zearelenone and deoxynivalenol (Marasas, Nelson & Toussoun, 1984) and it was demonstrated that deoxynivalenol (DON) can act as a virulence factor (McCormick, 2003). There is, therefore, a close relationship between disease severity and DON concentration.

*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. In the USA, Miller (1964) considers *F. graminearum* of prime importance as a soilborne pathogen of maize, but Hornby and Ullstrup (1967) only occasionally isolated *F. graminearum* from maize roots. The importance of the fungus as a crown and root rot pathogen under local conditions should be investigated in future research.

In the present study *P. terrestris* was significantly more frequently isolated at the first and second than the third sampling time (Table 7). Young and 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).

Except for *F. graminearum*, *F.oxysporum*, *P. terrestris* and *Trichoderma* spp., sampling time also affected the occurrences of *Acremonium* spp., *F. equiseti*, *F. nygamai*, *F. proliferatum* and *Pythium* spp (Table 7). *Acremonium* spp. and *F. equiseti* in crowns and *F. proliferatum* and *Pythium* spp. in roots increased significantly from the first to the third sampling times, and *F. nygamai* (not previously isolated from maize) incidence in roots and crowns decreased from the first to the third sampling time (Table 7). Chambers (1987a,b) isolated *Acremonium* spp. from maize roots and demonstrated that these fungi did not cause significant reduction in seedling emergence. *Fusarium equiseti* was also previously isolated from maize roots in South Africa by Smit *et al*., (1997) and was isolated at low frequencies from maize roots by Warren and Kommedahl (1973) in the USA. *Fusarium equiseti* does not appear to be an important pathogen of maize.

*Fusarium 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 or 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).

*Pythium* spp. isolated in this study included *P. aristosporum*, *P. irregulare*, *P. periilum*, *P. torulosum* and a few unidentified isolates. 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. aristosporum*, *P. arrhenomanes*, *P. graminicola*, *P. irregulare*, *P. paroecandrum*, *P. pulchrum*, *P. rostratum*, *P splendens*, *P. tardicrescens*, *P. torulosum*, *P. ultimum* and *P. vexans* (Zhang, Chen &Yang, 1998; Van Zeeland, Lamers & Van Dijk, 1999; 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; Scholte, 1987) 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. It is, however, important to note that *Pythium* infections are often limited to the feeder roots and it has been speculated that it is difficult to isolate *Pythium* spp., since feeder roots are often dislodged during digging and washing (White, 1999). In our study plant roots were surface disinfested before isolations, which could also have negatively affected the isolation of *Pythium* spp. from the roots, since Denman *et al.,* (1995) found that surface disinfestation significantly reduced the isolation frequency of *Pythium* spp. from lucerne roots.

The treatments applied in this trial significantly affected the incidence of *F. graminearum* (roots), *F. oxysporum* (roots), *F. proliferatum* (crown), *Pythium* spp. (roots) and *Trichoderma* spp. (crowns) (Tables 8 and 9). Methyl bromide treatment significantly reduced the incidence of *Pythium* spp. on roots compared to the other treatments. *Pythium* spp. were also significantly less frequently obtained from roots of Eco-T – and Extrasol-treated plots (Table 8). Studies conducted on wheat in the USA and South Africa showed that the increased yield responses of wheat following soil fumigation can be attributed to the control of *Pythium* root rot (Cook *et al*., 1987; Scott *et al*., 1992). Methyl bromide also significantly reduced the incidence of *F. oxysporum* in roots from the MB-treated plots compared to the Extrasol-treated plots, but not the control and Eco-T-treated plots. Certain fungi, such as *F. graminearum*, appeared to be favoured by the MB treatment. *Fusarium graminearum,* regarded as an important root rot pathogen of maize by Miller (1964), was isolated at significantly higher frequencies on roots from MB- and Extrasol- treated plots than from the control and Eco-T plots (Table 8). A significant location × treatment interaction occurred for *F. proliferatum* ($P = < .0001$) and *Trichoderma* spp. ($P = 0.0017$) isolated from crowns. Incidence of *F. proliferatum* was significantly higher in maize crowns at Bergville than at Winterton for all the treatments and was also significantly higher in crowns from the MB-treated plots at Winterton than the other treatments (Table 9). This is a very interesting phenomenon if it is taken into account that crowns and roots of the MB-treated plots were significantly more healthy

than the other treatments and that *F. proliferatum* is a known pathogen of maize (Kommedahl *et al.*, 1987). *Fusarium 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). Incidence of *Trichoderma* spp. was also higher in crowns from the MB plots at Bergville, but not at Winterton, where the highest incidence of *Trichoderma* in crowns was recorded for the Eco-T plots (Table 9).

**Table 8. Effect of treatment on the incidences of fungi in crowns and roots.**

| | | Incidence (%)[a] | | | |
|---|---|---|---|---|---|
| **Fungus** | **Plant Part** | **Control** | **Eco-T** | **Extrasol** | **Methyl bromide** |
| *Acremonium* sp. | Crown | 3.06a | 3.06a | 3.61a | 1.94a |
| | Root | 0.00a | 0.00a | 0.00a | 0.28a |
| *Fusarium equiseti* | Crown | 0.00a | 0.00a | 0.83a | 0.28a |
| | Root | 1.39a | 1.67a | 2.22a | 3.89a |
| *Fusarium graminearum* | Crown | 1.67a | 0.56a | 3.06a | 1.39a |
| | Root | 16.17 | 16.11 | 24.17 | 25.00 |
| *Fusarium nygamai* | Crown | 0.83a | 1.39a | 1.94a | 0.00a |
| | Root | 2.50a | 2.78a | 4.17a | 3.06a |
| *Fusarium oxysporum* | Crown | 3.89a | 5.28a | 6.39a | 5.83a |
| | Root | 27.50ab | 29.17ab | 33.61a | 21.11b |
| *Fusarium proliferatum* | Crown | -[b] | - | - | - |
| | Root | 2.22a | 2.22a | 2.50a | 4.17a |
| *Fusarium solani* | Crown | 0.28a | 0.56a | 0.28a | 0.83a |
| | Root | 8.61a | 5.83a | 4.17a | 4.72a |
| *Fusarium subglutinans* | Crown | 1.11a | 1.11a | 0.28a | 1.67a |
| | Root | 0.28a | 0.83a | 1.39a | 2.22a |
| *Pyrenochaeta terrestris* | Crown | 0.56a | 0.28a | 1.67a | 0.56a |
| | Root | 7.50a | 9.72a | 8.33a | 6.94a |
| *Pythium* spp. | Crown | 0.00a | 0.00a | 0.00a | 0.00a |
| | Root | 7.07a | 3.93b | 3.89b | 0.00c |
| *Trichoderma* spp. | Crown | - | - | - | - |
| | Root | 43.61a | 42.78a | 31.39a | 35.83a |

[a] Means within a fungus within a row followed by the same letter do not differ significantly (P = 0.05)
[b] - See Table 9 for data on significant interactions

**Table 9. Effect of location and treatment on the incidence of fungi on crowns of maize plants.**

| Fungus | Location | Incidence (%)[a] Control | Eco-T | Extrasol | Methyl Bromide |
|---|---|---|---|---|---|
| *Fusarium proliferatum* | Bergville | 1.11de | 0.00e | 0.00e | 0.00e |
| | Winterton | 5.56b | 3.89bc | 2.78cd | 14.44a |
| *Trichoderma* spp. | Bergville | 7.22b | 5.56b | 6.67b | 18.89a |
| | Winterton | 5.56b | 17.22a | 8.33b | 1.11b |

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

Incidences of *F.solani* and *F. subglutinans*, were not significantly affected by the locality, sampling time or treatments (Tables 5, 7 and 8). *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). Similarly *F. subglutinans* is not listed as an important root pathogen of maize.

*Rhizoctonia* spp. were infrequently isolated from crowns and roots in this study and isolates were not identified to species and anastomosis group level. *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.

Plant parasitic nematodes can significantly reduce maize yields. Martin, Way and Armstrong (1975) demonstrated that fumigation of sandy soils in Zimbabwe with ethylene dibromide (EDB) reduced populations of *Pratylenchus brachyurus* and *P. zeae* and increased maize yields by up to 33%. In South Africa, Walters (1979) reported that MB fumigation to control *P. zeae* and *Trichodorus* on maize gave a 128% yield increase.

In addition to quantifying disease severity and isolating fungi from diseased roots, we also sampled one plant from each plot as a preliminary screening for nematode incidence. *Helicotylenchus dihystera*, *H. paraplatyurus*, *H. pseudorobustus*, *H. vulgaris*, *Meloidogyne* sp., *Paratrichodorus minor*, *P. zeae*, *Rotylenchus parvus*, *Scutellonema brachyurus* were recorded on the plant roots and soil from Bergville and Winterton and there are indications that the MB fumigation reduced the numbers of plant parasitic nematodes. More detailed investigations are, however, needed before the importance of these nematodes in reducing maize yield can be determined. It is also important to note that interactions between nematodes and soilborne pathogens are important (Palmer & McDonald, 1974). Although Chambers (1987a) could not find any association between *Pratylenchus* spp. and root rot, Jordaan *et al*., (1987) found that nematodes and *F. verticillioides* affected plant growth more when combined than when alone. It is, therefore, possible that nematodes interacted with the soilborne pathogens to cause root damage at our trials in Bergville and Winterton and this should be investigated in future research.

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). 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". Krüger and Speakman (1997) in Germany, found that monoculture maize led to a high level of root rot after about four 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, 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.

A considerable amount of information is available on the effect of conservation tillage on ear and stalk rot pathogens of maize. In South Africa, Flett, McLaren and 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 and Lipps (1996) that *P. arrhenomanes* is favoured by poorly drained soil when continuous maize cropping and no-till are practised. Crop rotation is clearly very important in no-till maize production systems and Howard, Chambers and Lessman (1998) found that maize yields were increased 14% and soyabean yields 11% with rotations. Since conservation tillage is increasingly promoted in South Africa, it is essential to determine the combined effects of crop rotation and conservation tillage on soilborne diseases of maize if yield losses are to be minimized.

**Estimation of microbial activity.** Pre-plant samples were taken during December 2005 to act as baseline for this and possible future studies. Although community level physiological profiles (CLPP) from four stages (pre-plant, seedling stage, mid-season and soft dough) were analysed for functional diversity, only results from the last sampling time are discussed in detail. Erratic results obtained during the second and third sampling times could be attributed to extremely wet soil samples, which might have had a negative effect on oxygen supply, thus influencing the survival of bacteria during transport and storage (Brady, 1974).

**Functional diversity**

***Community level physiological profiles*.** During the third sampling time, a clear distinction could be made between carbon source utilisation patterns of soil microbial populations at Bergville (F) and Winterton (M) (Fig. 9). This could be attributed to soil microorganisms with different carbon source utilisation patterns at the two localities (Ritz *et al*., 2003). Only molecular analyses (not conducted in this study) could clarify if and to what extent the composition of the populations differs.

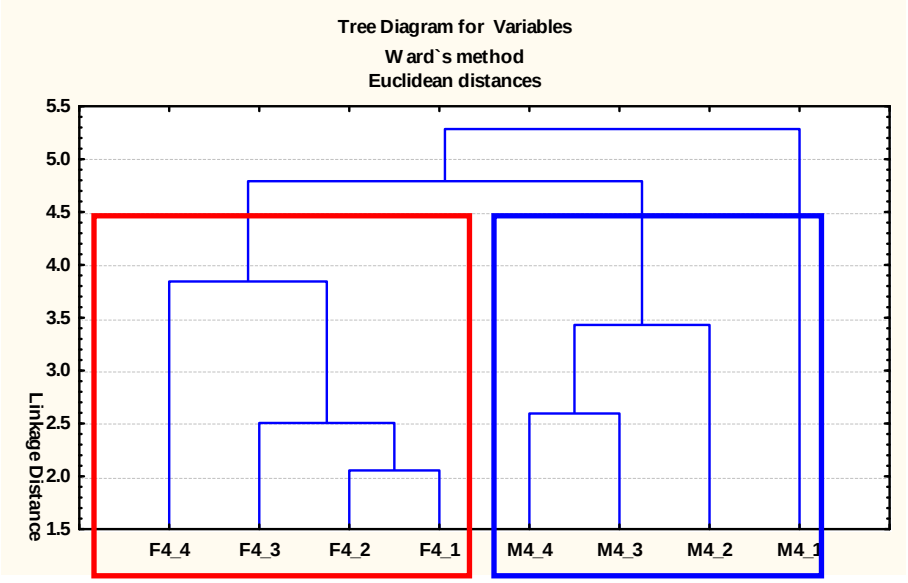
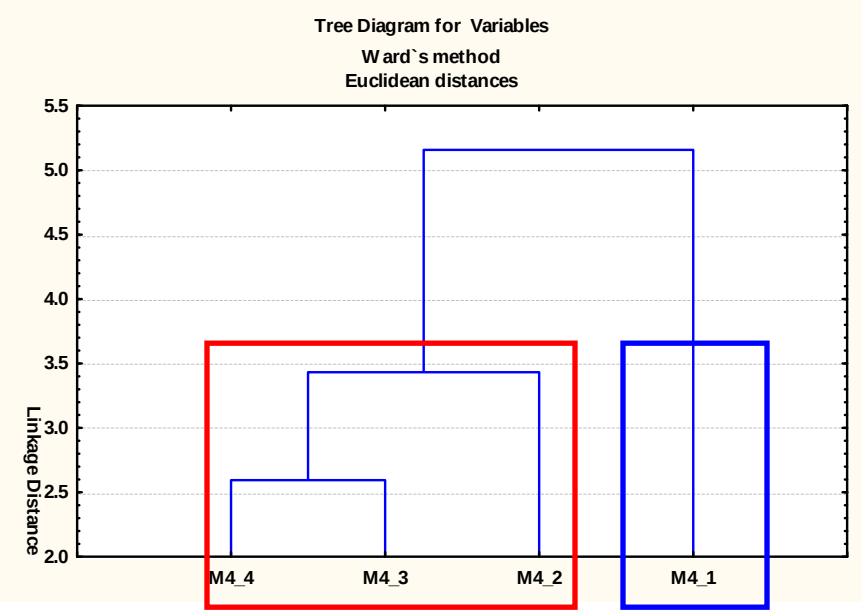



**Fig. 9. Dendogram illustrating the difference in carbon source utilisation patterns between the Bergville (F) and Winterton (M) localities. Methyl Bromide (4_1); Control (4_2); Eco-T (4_3); Extrasol (4_4).**

**(a)**




**(b)**

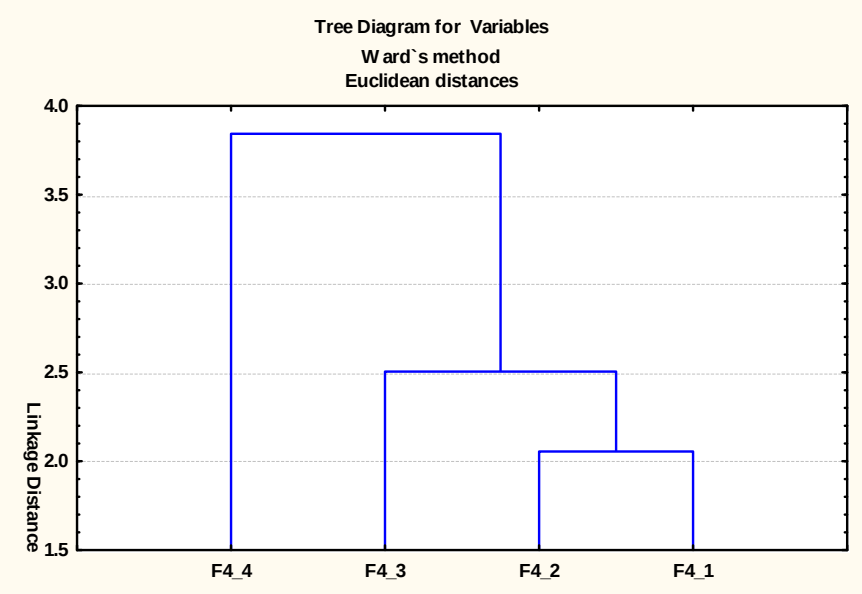
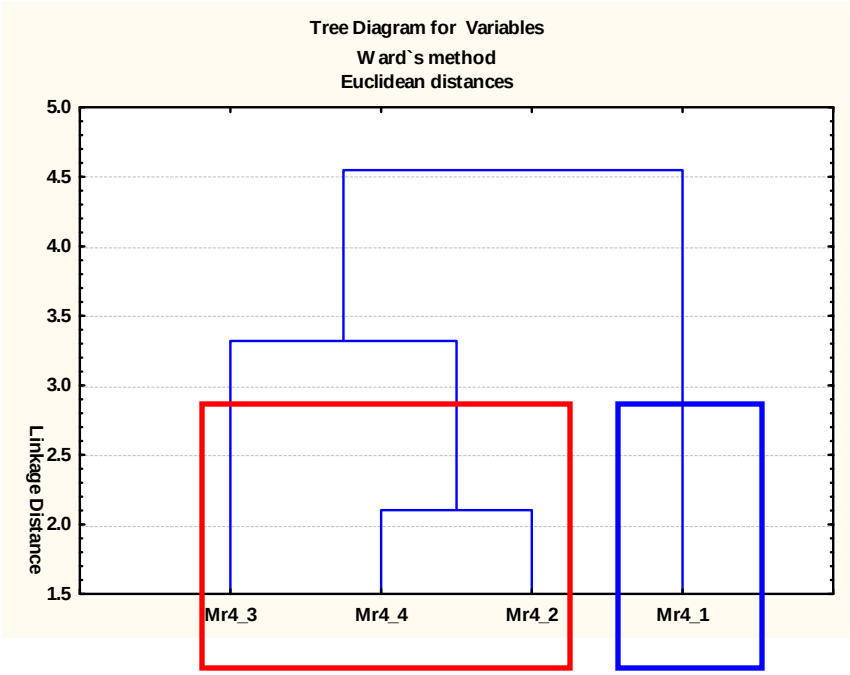



**Fig. 10. Dendogram illustrating the difference in carbon source utilisation patterns between the MB treatment and the other treatments at (2a) Winterton (M) and (2b) Bergville (F) localities. Methyl Bromide (4_1); Control (4_2); Eco-T (4_3); Extrasol (4_4).**

A clear dissimilarity exists between the carbon source utilization patterns of soil microorganisms in the MB treatment and the other treatments at Winterton (Fig. 10a). This clear distinction was not observed, however, between treatments at Bergville (Fig. 10b). When the soil in direct contact with the rhizosphere was analysed, clear dissimilarities were observed between carbon source utilisation profiles in MB and those present in other treatments at Winterton (M) (Fig. 11a) and Bergville (F) (Fig. 11b). These dissimilarities were not statistically significant, but could be attributed to the in-row applications of fertilizer, Eco-T and Extrasol. The in-row applications could have influenced maize root activities, thus having a greater effect on soil microbial activity in direct contact with the rhizosphere, compared to soil microbial activity in bulk soil. Clear discrimination has also been found between carbon sources utilised by microbial communities from different plant rhizospheres by Grayston *et al*., (1998). The ambiguous distinctions observed when analysing bulk soil in contrast to soil directly in contact with the rhizosphere, could also be attributed to possible cross-contamination between treatments during soil sampling.

**(a)**




**(b)**

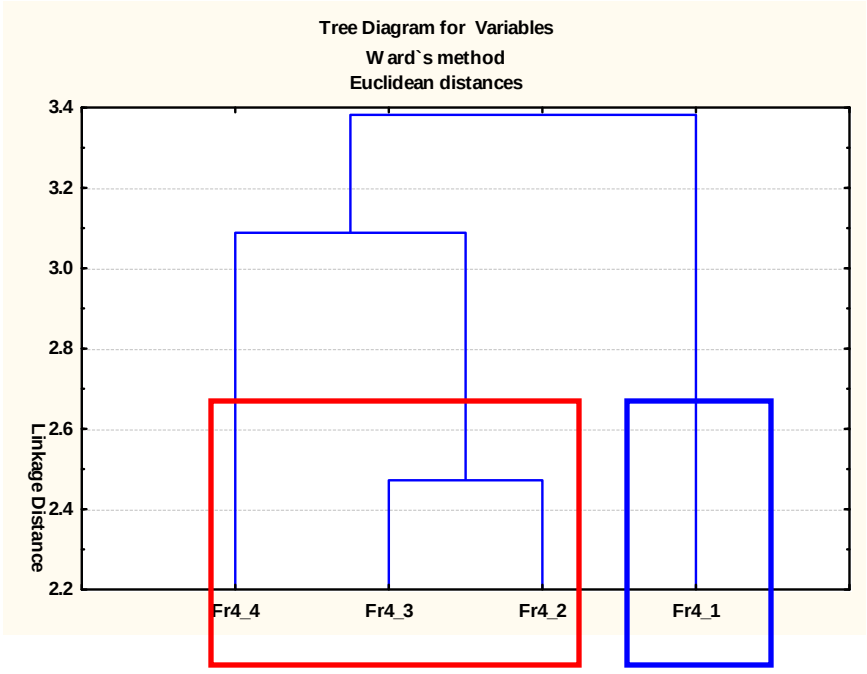



**Fig. 11.** **Dendogram illustrating the difference in carbon source utilisation patterns between the MB treatment and the other treatments at (3a) Winterton (M) and (3b) Bergville (F) localities during analyses of soil in direct contact with rhizosphere. Methylbromide (4_1); Control (4_2); Eco-T (4_3); Extrasol (4_4).**

***Diversity indices.*** Shannon-Weaver substrate diversity indices distinguish soil populations based on the number of different carbon sources utilised. According to Magurran (1988), values of the index range between 1.5 and 3.5 and rarely increase above 4.5. Values obtained at both Winterton (Fig. 12a) and Bergville (Fig. 12b) were within the intermediate diversity range (2.4 – 2.9). The substrate diversity values could be attributed to the moderate percentage of carbon sources utilized (results not shown).

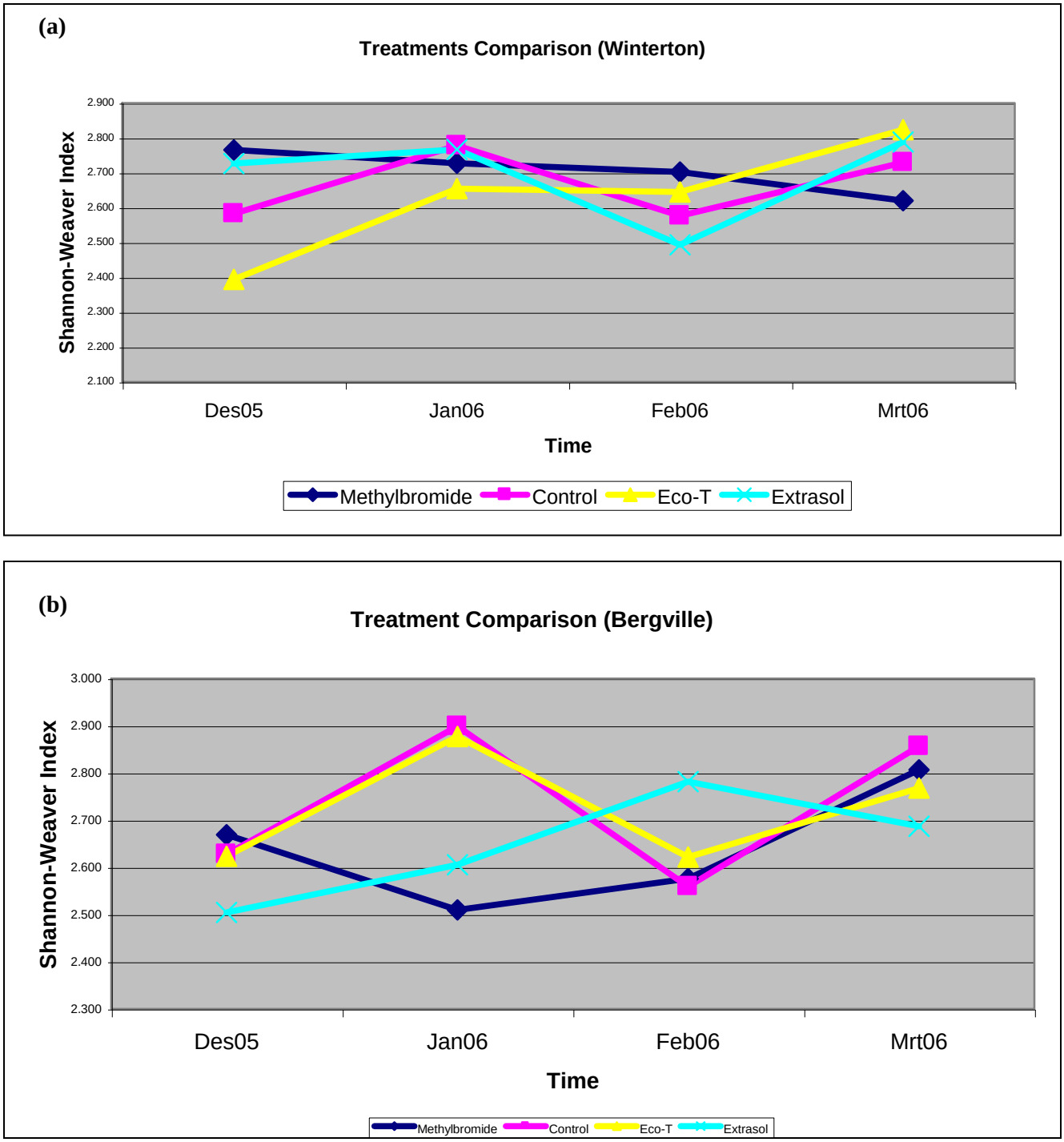
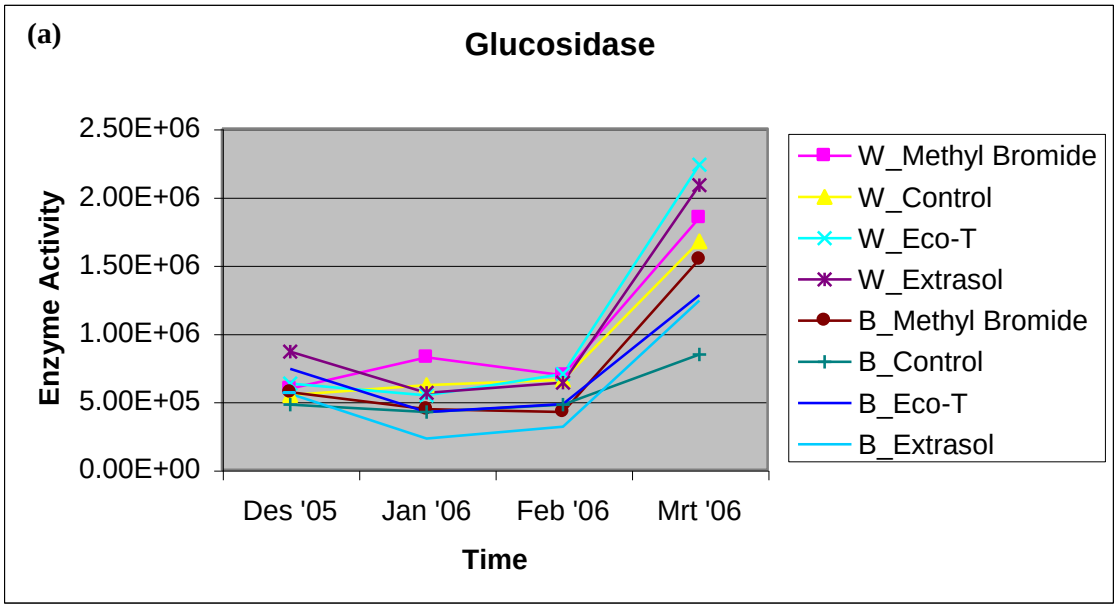


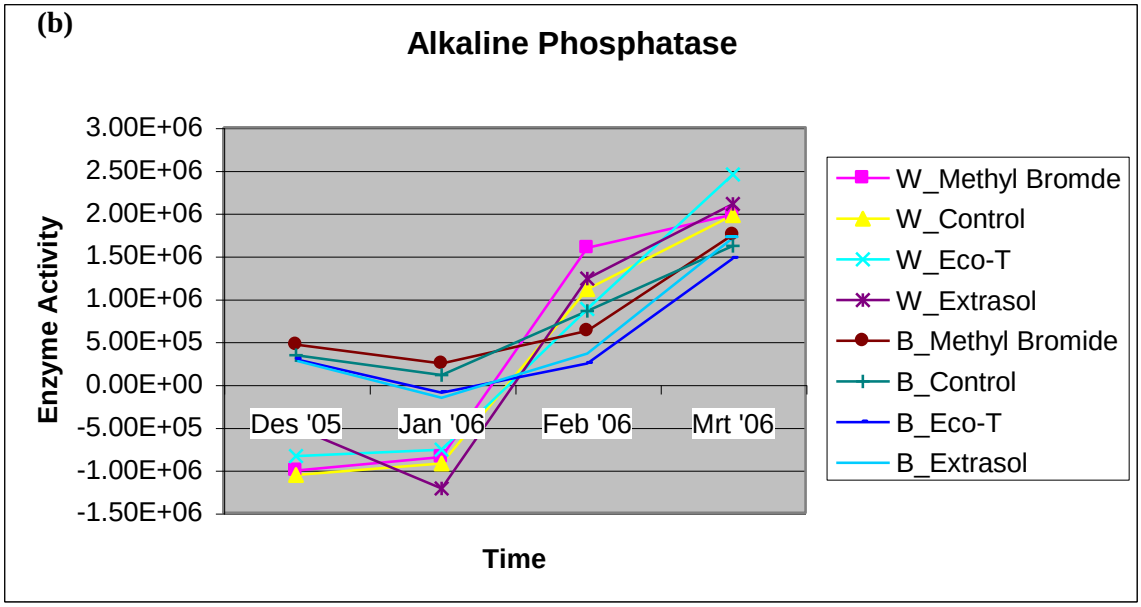

**Fig. 12. Shannon-Weaver substrate diversity index values obtained for (4a) Winterton and (4b) Bergville.**

Microbial diversity in both localities' control treatments followed the same trend over the sampling times. The trend indicates an increase in microbial diversity, but fluctuations during first and second samplings times could be attributed to particularly wet conditions that might have had a negative effect on oxygen supply (Brady, 1974). 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. 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 (Garland, 1996).

In contrast to the other treatments, microbial diversity in the MB treatment at Winterton decreased over time. Crop residues were incorporated prior to fumigation, and this might have attributed to a decline in microbial diversity over time. Since the composition of plant root exudates differ between plant types (Garland, 1996), leakage of maize root exudates might stimulate particular rhizosphere microorganisms that are especially well adapted to utilise the maize root exudates very rapidly (Duffy & Weller, 1995). The difference in soil microbial diversity between the two localities, on the other hand, might be ascribed to the fact that soil microbial populations at Bergville have not yet stabilised after approximately 10 years of no-till and periodic maize trash burning, and could thus be more susceptible to perturbation compared to Winterton (> 20 years of no-till).

**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 (Fig. 13a), alkaline phosphatase (Fig. 13b), acid phosphatase (Fig. 13c), and urease (Fig. 13d) activities within the topsoil of the two localities were assayed because of their vital role in soil microbial activity and organic C, N and P mineralisation (Dick, 1997; Deng & Tabatabai, 1997).

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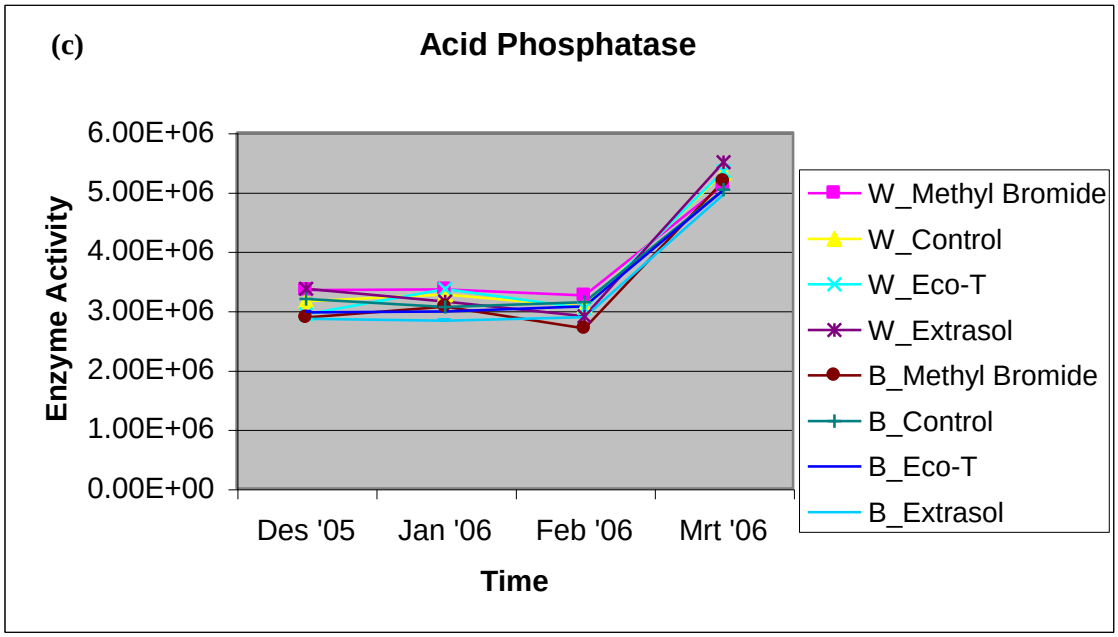


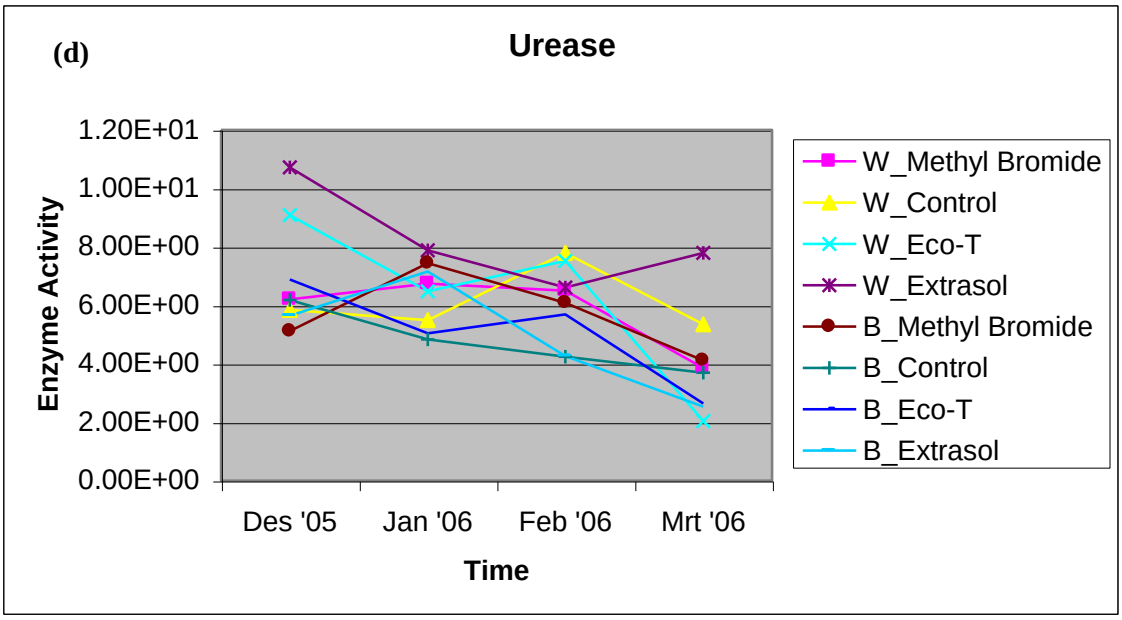


**Fig. 13. ß-Glucosidase (5a), alkaline phosphatase (5b), acid phosphatase (5c), and urease (5d) activity for Winterton and Bergville over a period of four months.**

Although initial soil enzyme activities during pre-plant sampling (December 2005) were relatively low, a gradual increase in enzyme activity could be observed over time (Fig. 13a-c) due to stimulation of microbial activity (Martens *et al.*, 1992) through cropping systems that return crop residues to the field (Jordan *et al*., 1995). Bolton *et al*., (1985) also found that crop rotation had a positive effect on soil enzyme activities. 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). 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 (Fig. 13d). Treatments at Winterton had a slightly higher ß-glucosidase (Fig. 13a) and alkaline phosphatase (Fig. 13b) activity than Bergville. This might be ascribed to crop rotation and no-till practices over long periods of time, which increased soil enzyme activity by stimulating microbial activity (Martens *et al*., 1992).

## CONCLUSIONS

This investigation clearly demonstrated the effects of soilborne pathogens on growth and yield reduction in maize following winter wheat in no-till systems in KwaZulu-Natal. It was shown that the treatments that were evaluated and the tillage of soil prior to the application of MB had no influence on plant nutrition and that growth and yield responses were the result of other benefits, possibly enhanced moisture recovery, associated with improved root health.

Significant improvement in crown and root health, growth and grain yield was especially evident at Winterton. Except for the differences in soil types at the two locations, freely drained Hutton soil at Winterton and a shallower wetter Avalon soil at Bergville, plants in the fumigated plots at Bergville also displayed severe symptoms of metolachlor toxicity. This could possible account for the fact that differences between the MB treatment and the other treatments at Bergville were not always as significant as at Winterton.

The significant increases in growth and grain yield that were obtained in plots fumigated with MB is noteworthy**,** since the trial was conducted under near optimal conditions with regard to moisture and nutrient availability. Under suboptimal conditions one would expect differences in growth and yield of plants from fumigated and non-fumigated plots to be even more pronounced, given the severity of crown and root rot that occurred.

The two biocides tested in this study did not differ significantly from the control with regard to growth and grain yield. Incidence of *Pythium* spp. on roots from the Eco-T and Extrasol plots was significantly lower than the control, but in general these treatments did not differ from the control with regard to other pathogenic fungi isolated.

Our results confirmed those obtained by other researchers on the usefulness of soil fumigation with MB as an experimental tool to demonstrate the effects of soilborne pathogens on crown and root health. Although soil fumigation with MB would never be a commercial option for maize farmers to use, it demonstrates the potential yield of a healthy crop. These data can then be used in crop rotation trials to determine the most profitable and sustainable rotation system.

In this trial, crown and root rot seem to have been caused by a complex of fungi and possibly also nematodes. The most important fungal pathogens frequently isolated from diseased crowns and roots were *F. graminearum*, *Pyrenochaeta terrestris* and *Pythium* spp., but their relative importance in causing crown and root rot is not clear at this stage. Incidence of *F. graminearum* and *Pythium* spp increased during the growing season, whereas incidence of *P. terrestris* was significantly lower at the end than at the beginning of the season. It is clear that there is a succession of pathogenic fungi during the growing season, which agrees with findings of researchers in other countries. Information on the succession of soilborne pathogens in maize production will be invaluable in the development of management strategies against soilborne diseases of maize.

Soil fumigation significantly reduced the incidence of *Pythium* spp. and also tended to reduce that of *P. terrestris.* Incidence of *F. oxysporum*, the second most prominent fungus isolated, but generally regarded as a weak pathogen of maize, was also significantly reduced by soil fumigation. However, *F. graminearum* was more frequently isolated from the MB-treated plots than from the other treatments. The high incidence of *F. graminearum* in the roots from the MB plots is difficult to explain, but it is possible that the fungus was spread with rain water or during sampling of the fumigated plots, as being an aggressive pathogen, the fungus could easily recolonize the 'sterile' soil. The severity of crown and root rot increased significantly in all the treatments from the first sampling to the third sampling and lesions were more frequently observed on roots from the fumigated plots at the third sampling time. The effect of fumigation was more pronounced at the beginning than at the end of the season and it is possible that the later infection of crowns and roots in the MB-treated soil did not have such a pronounced effect on the yield.

This study demonstrated the effect of wheat-maize rotations on the incidence of *F. graminearum*, with an average of 41% of pieces of diseased tissue yielding the fungus at the third sampling time. The trial did not evaluate the effect of crops other than wheat, but clearly identified the need for such studies. The weather conditions in KZN are particularly favourable for infection of maize and wheat with *F. graminearum*, which is primarily

regarded as a stalk and ear rot pathogen of maize and the causal agent of scab on wheat. The fungus also produces mycotoxins which are harmful to animals and humans.

The high incidence of *F. proliferatum* in crowns of plants from fumigated soil at Winterton is interesting. *Fusarium proliferatum* has been shown to be a weak pathogen of maize compared to *F. graminearum* and there is a possibility that *F. proliferatum* infection of the roots and crowns protected plant crowns from infection by *F. graminearum.* Considering the importance of *F. graminearum* as a pathogen of maize, this phenomenon should be investigated in future research.

*Trichoderma* spp. were the most predominant fungi isolated from crowns and roots in this study. This confirms the reports of other researchers that *Trichoderma* spp. occur frequently on maize tissue. The *Trichoderma* isolates that were obtained were not identified to species level due to financial constraints and it is, therefore, not possible to determine the impact of the different treatments, localities and sampling times on the different species. The identification of species of *Trichoderma* associated with maize roots is important for future research, since *Trichoderma* has been listed as both a pathogen of maize and a biocontrol agent.

Plant parasitic nematodes were isolated from the maize roots and rhizosphere soil and there are indications that soil fumigation reduced the nematode numbers in comparison with the other treatments. Although the identification of nematodes associated with maize roots was not part of the original project proposal, this preliminary investigation and the fact that other studies have reported on interactions between soilborne fungi and nematodes on maize, indicated that future research should include an evaluation of the effect of different treatments, such as crop rotation, on plant parasitic nematodes associated with maize roots and soil.

The effect of soil fumigation on microbial diversity in the soil was demonstrated by the carbon utilization profiles, which separated the soil treated with MB from the soils subjected to the other treatments. The results were inconsistent when wet soil samples were used and it was shown that it is important to use rhizosphere soil to detect the effect of treatments on functional diversity of microorganisms in soil.

Analysis of enzyme activity showed more activity in the Winterton than the Bergville soil. Since enzyme activity is an indicator of soil degradation, it is interesting that the Winterton soil, where crop rotation and no-till have been practiced for 20 years compared to the 10 years at Bergville, had higher enzyme activities. The difference in enzyme activity in the Winterton and Bergville soils conceivably contributed to the significantly higher yields obtained in the Winterton trial. It is also important to note that the microbiological techniques used in this study were both cost-effective and useful as soil quality markers.

Finally, it is perhaps pertinent to emphasize the wider implications of this preliminary study. Valuable information has been obtained regarding the incidence and spectrum of soilborne diseases in maize rotated with winter wheat and the effects of these diseases on final grain yield. The challenge now is to establish the effects of possible alternative rotations and ameliorative procedures. Such information is very limited in South Africa and the relatively few rotational studies that have been carried out with maize have seldom directly addressed the effects on soilborne diseases and microbial diversity *per se.* There is a pressing need to determine the impact of crops other than wheat and of biocontrol agents on soilborne pathogens of maize and on microbial activity and diversity in the soil. Only once suitably detailed studies have been carried out will it be possible to develop optimal intervention strategies. The eventual impact on maize yield ceilings and agricultural sustainability is likely to be appreciable.

**ACKNOWLEDGMENT**

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