

Final Report

**THE ROLE AND IMPORTANCE OF SOILBORNE
DISEASES AND THEIR INTEGRATED
MANAGEMENT IN SUSTAINABLE MAIZE
PRODUCTION**

FACET 1

**THE ROLE AND IMPORTANCE OF SOILBORNE DISEASES AND MICROBIAL
DIVERSITY AND ACTIVITY IN MAIZE PRODUCTION AS AFFECTED BY CROP
ROTATIONS, BIOCONTROL PRODUCTS, CHEMICAL BIOCIDES, SOIL
DISTURBANCE AND RESIDUE COVER**

FACET 4

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

**Submitted on behalf of
the No-Till Club of KwaZulu-Natal**

September 2010

LIST OF CONTENTS

THE ROLE AND IMPORTANCE OF SOILBORNE DISEASES AND THEIR INTEGRATED MANAGEMENT IN SUSTAINABLE MAIZE PRODUCTION

Description	Page
FACET 1	
THE ROLE AND IMPORTANCE OF SOILBORNE DISEASES AND MICROBIAL DIVERSITY AND ACTIVITY IN MAIZE PRODUCTION AS AFFECTED BY CROP ROTATIONS, BIOCONTROL PRODUCTS, CHEMICAL BIOCIDES, SOIL DISTURBANCE AND RESIDUE COVER	1
EXECUTIVE SUMMARY	1
INTRODUCTION	7
OBJECTIVES	10
MATERIALS AND METHODS	10
STATISTICAL ANALYSES	17
RESULTS AND DISCUSSION	18
General information on parameters measured	18
Crown and root rot severity	19
Fungi associated with crowns and roots	19
Nematodes	23
Microbial diversity and activity in soil	30
ROTATIONAL EFFECTS	30
Growth, plant and soil analysis	30
Crown and root rots	40
Fungi associated with crowns and roots	42
Nematodes	46
Microbial diversity and activity in soil	46

	Description	Page
BIOCONTROL AGENT EFFECTS	49
Growth, plant and soil analysis	49
Crown and root rots	50
Fungi associated with crowns and roots	50
Nematodes	50
Microbial diversity and activity in soil	52
CHEMICAL BIOCIDES EFFECTS	55
Growth, plant and soil analysis	55
Crown and root rots	56
Fungi associated with crowns and roots	56
Nematodes	57
Microbial diversity and activity in soil	58
TILLAGE EFFECTS	60
Growth, plant and soil analysis	60
Crown and root rots	61
Fungi associated with crowns and roots	62
Nematodes	63
Microbial diversity and activity in soil	64
WHEAT STRAW COVER EFFECTS	67
Growth, plant and soil analysis	68
Crown and root rots	75
Fungi associated with crowns and roots	77
Nematodes	78
Microbial diversity and activity in soil	79
SUMMARY AND CONCLUSIONS	82
ROTATIONAL EFFECTS	82
BIOCONTROL AGENT EFFECTS	83
CHEMICAL BIOCIDES EFFECTS	83
TILLAGE EFFECTS	84
WHEAT STRAW COVER EFFECTS	85

Description	Page
FACET 4	
THE RELATIVE IMPORTANCE OF FUNGI FREQUENTLY ASSOCIATED WITH DISEASED MAIZE CROWNS AND ROOTS AS SOILBORNE PATHOGENS OF MAIZE AND ROTATION CROPS, AND THE INTERACTION BETWEEN FUNGAL PATHOGENS AND PARASITIC NEMATODES ON MAIZE	92
EXECUTIVE SUMMARY	92
INTRODUCTION	93
MATERIALS AND METHODS	95
RESULTS	96
Summary of pathogenicity tests	113
CONCLUSIONS	113
REFERENCES	116

FACET 1

THE ROLE AND IMPORTANCE OF SOILBORNE DISEASES AND MICROBIAL DIVERSITY AND ACTIVITY IN MAIZE PRODUCTION AS AFFECTED BY CROP ROTATIONS, BIOCONTROL PRODUCTS, CHEMICAL BIOCIDES, SOIL DISTURBANCE AND RESIDUE COVER

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EXECUTIVE SUMMARY

The overall objective of this project is to establish practical and economic ways of reducing the yield-limiting effects of soilborne diseases in no-till systems of maize production. Specific objectives of this facet are to (1) assess the effects of pathogenic fungi and parasitic nematodes on the growth of maize following winter wheat, (2) identify and quantify the species involved, (3) examine the effects of alternative winter rotational crops, (4) measure the ameliorative effects of biological control products marketed to farmers and of potential chemical biocides, (5) compare soil disturbance after winter wheat in the place of no-till, (6) establish the importance and role of surface cover in disease suppression and yield, and (7) determine the influence of these potentially ameliorative strategies on microbial activity and diversity in the soil.

This multidisciplinary project employs skills from specialists in plant pathology, nematology, soil microbiology, soil science and agronomy that are based at the ARC-PPRI in Stellenbosch and Pretoria, the KZN Department of Agriculture (Cedara College) and Omnia Fertilizers in Howick (KZN). Funding for this project is provided by the Maize Trust, KZN Department of Agriculture and Omnia Fertilizers.

The trial is located in the Winterton district on the farm of Mr Anthony Muirhead and consists of 19 treatments, which included the winter crops canola (CAN), crambe (CR), black oat (BO), stooling rye (SR), wheat without tillage (C), maize fallow (FM) and bare fallow (BF); chemical soil treatment with anhydrous ammonia (ANM) and

methyl bromide (MBM); biocontrol products Eco-T (ECO), Gliogrow (GLIO), Fungimax + Organoboost (OB) and a combination of OB and anhydrous ammonia (AN+OB); disturbance of soil with ripping (CRI) and tillage (TM), and application of wheat straw cover on chemically treated (ANP & MBP) and fallow (FP) and tillage (TP) plots. The maize cultivar PHI 32D96B was used and weeds and foliar diseases were chemically controlled. Above-ground biomass yields were determined 28, 64 and 140 days after planting (DAP). Plants sampled 28 DAP and leaf samples (71 DAP) were analysed for nutrient composition. Soil temperature and moisture content were determined 42, 73 and 135 DAP and earthworm counts were conducted 73 and 135 DAP. Root lodging was recorded 140 DAP and soil infiltration rates were determined 154 DAP. Disease assessments, microbial analyses and analyses of rhizosphere soil were done 64 and 140 DAP and nematode analyses in soil and roots 64 DAP. Maize was harvested 170 DAP.

The highest yields (15.7 t/ha) were recorded for the anhydrous ammonia plus extra straw cover (ANP) and the lowest (12.5 t/ha) for the bare fallow (BF) treatments. At both 73 and 135 DAP the highest earthworm counts were recorded for the black oat (BO) treatment and the lowest for the bare fallow treatment. Earthworm counts were significantly negatively correlated with crown and root rot severity 140 DAP. The highest soil moisture and temperature were recorded for fallow plus extra cover (FP) and anhydrous ammonia without extra cover (ANM), respectively, and the lowest soil moisture and temperature were recorded for tilled without extra cover (TM) and black oat (BO), respectively, 73 DAP. At 135 DAP the ripped treatment (CRI) had the highest temperature and FP the highest soil moisture. TM had the lowest soil moisture content and MBP the lowest temperature. The BF treatment had the slowest and the TP and ANP treatments the fastest infiltration rates. The tilled and methyl bromide treatments without extra straw cover (TM and MBM) had the highest number of plants lodged and the control treatment the lowest. The highest amount of soil microbial species was observed in the OB treatment, with chemical-biocide and fallow treatments without added cover demonstrating the lowest amount of microbial species. The highest overall microbial enzyme activity was observed in stooling rye treatments, whereas methyl bromide treatments produced the lowest overall enzyme activity. The highest crown and root rot severities were recorded for the bare fallow (BF) treatment and the lowest for the methyl bromide treatment with extra straw cover (MBP). Crown and root rot severity were significantly positively correlated 64 DAP and 140 DAP. There were also significant negative correlations between crown and root rot severity 140 DAP and grain yield 170 DAP.

Fungi most frequently isolated from crowns and roots were *Acremonium* spp., *Diplodia/Stenocarpella* spp., *Fusarium equiseti*, *F. graminearum*, *F. oxysporum*, *F. proliferatum*, *F. solani*, *F. subglutinans*, *Phialophora* spp., *Pyrenochaeta terrestris* and *Trichoderma* spp. Incidences of *Phialophora* spp. in roots 64 DAP and of *Diplodia/Stenocarpella* spp. in roots 140 DAP were significantly negatively correlated with grain yield. Incidences of *F. graminearum* in crowns 140 DAP were significantly positively correlated with lodging. Significant sampling time x treatment interactions were recorded for *F. graminearum* (crown) and *Phialophora* spp. (crown).

Herbivorous nematodes (plant-parasitic nematodes) extracted from the soil and roots were *Criconeoides sphaerocephalus*, *Dorylaimellus* species, *Helicotylenchus dihystrera*, *Longidorus pisi*, *Meloidogyne* species, *Paratrichodorus minor*, *Pratylenchus brachyurus*, *Pratylenchus zaeae*, *Rotylenchulus parvus*, *Scutellonema brachyurus*, *Scutellonema unum* and *Tylenchorhynchus brevilineatus*. All these nematodes were previously reported from maize in South Africa and as herbivores can inflict qualitative damage to underground plant parts but the damage can be in a less direct way in that the growth and development of attacked plants is reduced. The members of the family Hoplolaimidae and the *Pratylenchus* species reached the highest population numbers. The lowest incidence of herbivores was recorded in the methyl bromide treatments and the highest in the canola treatment. For the first time the beneficial nematodes were separated into the different feeding types and the correlation between these nematodes determined. Almost all the treatments benefited the herbivores and the incidence of the bacterivores was lower than that of the fungivores in only the anhydrous ammonia with cover and the tilled treatments. A very positive aspect observed during the current season was the continuing increase in the incidence of beneficial nematodes.

Rotation of maize with the winter crops, black oat, canola, crambe and stouling rye significantly reduced crown and root rot severity in maize 64 DAP planting, and 140 DAP root rot in maize following canola, black oat and stouling rye was reduced. Maize fallow (FM) had significantly less root rot 64 DAP than the control (wheat), but not 140 DAP. The highest crown and root rot severities were recorded for the bare fallow (BF) treatment 140 DAP. Incidences of *F. graminearum* in roots of maize planted after bare fallow, canola, crambe and fallow without cover were significantly lower than in roots of maize planted after black oat, wheat and stouling rye. Although no significant yield benefits were evident including rotation with the winter crops 140 DAP, and at harvest, canola and crambe resulted in increased yields 28 and 64 DAP and stouling rye significantly increased yields 64 DAP. Maize fallow and bare fallow significantly increased yields 28 DAP, due probably to higher soil

temperatures in these treatments during early growth. At 64 DAP, no benefits due to the fallow treatments were evident and 140 DAP and harvest yields in these treatments were markedly lower than in control plots. Rotational treatment had no significant affect on herbivorous nematodes. The total number of herbivores in most of the rotational treatments was significantly more than in the maize after wheat treatment. In both the black oat and canola treatments the population numbers of the bacterivorous nematodes were significantly higher than that of the fungivores. These two treatments benefited bacterivorous over fungivorous nematodes.

The biocontrol products, Eco-T (ECO), Gliogrow (GLIO) and Fungimax plus Organoboost (OB) significantly reduced crown rot severity 64 DAP, but only Gliogrow reduced root rot 64 DAP. At 140 DAP these benefits were not significant anymore. At neither grow stage was crown and root rot severities for the ANM+OB different from OB treatment. The biocontrol products benefitted herbivorous nematodes 64 DAP, did not result in significant yield differences at any stage, and also did not reduce *F. graminearum*, *Diplodia/Stenocarpella* spp and *Phialophora* spp. in crown and roots, ANM+OB and GLIO significantly increased soil microbial species dominance with microbial communities between 64 and 140 DAP. *Trichoderma* spp. were more frequently associated with Eco-T and this treatment indicated a slightly higher potential to increase soil quality and fertility.

The chemical biocides, anhydrous ammonia (ANM) and methyl bromide (MBM), significantly reduced crown and root rot 64 DAP, but not 140 DAP. These treatments also significantly reduced incidences of *F. graminearum* in maize roots. Anhydrous ammonia significantly increased plant Mn content and also significantly increased yields 64 DAP, but not 28 and 140 DAP. At harvest, the yield benefit of anhydrous ammonia was highly significant. Methyl bromide fumigation significantly increased yields 28 DAP, but this was possibly due to increased soil temperatures during early growth. At 64 DAP no differences in yield relative to the control were evident and 140 DAP and at harvest the effects of methyl bromide minus extra cover were significantly negative. Lower soil moisture content and reduced earthworm populations were probably implicated. The lowest number of herbivorous nematodes was observed in the methyl bromide treatments, and this treatment (MBM) was one of two treatments where the incidence of the beneficial nematodes was lower than that of the previous season. Significantly more *Trichoderma* spp. were associated with crowns and roots of the MBM treatment, but this treatment demonstrated the lowest amount of soil microbial species and overall enzyme activity and the highest level of species dominance within the soil microbial community.

Tillage very markedly increased the prevalence of root lodging. Reduced soil moisture content in these treatments, higher temperatures and reduced earthworm populations were probably implicated. Tillage (TM) and ripping (CRI) significantly reduced crown and root rot 64 DAP, but at 140 DAP the TM treatment had significantly more crown and root rot than CRI and the control (C). At 140 DAP the MBM (tilled + methyl bromide) and ANM (ripped + anhydrous ammonia) had consistently less root rot than just tilled (TM) and ripped (CRI) treatments. Soil disturbance significantly reduced incidences of *F. graminearum* in roots, but not crowns. Highest incidences of this fungus and *Phialophora* spp. in crowns were recorded for the TM treatment, which demonstrated high root lodging. Tillage (rotovation or ripping) significantly increased yields 28 DAP, due probably to increased soil temperatures during early growth. These effects were not evident at later samplings, however, and in the absence of extra cover, tilled plots were significantly out-yielded by the control. Plots ripped without anhydrous ammonia yielded over 2000 kg/ha less than their analogues that received gas. The highest population numbers of fungivorous nematodes were found in the tilled minus cover treatment which was one of the few treatments where the beneficial nematodes and not the herbivorous nematodes, were benefited. The ripped and tillage treatments without cover insignificantly decreased the number of different bacterial species. The CRI treatment resulted in the highest alkaline phosphatase activity, whereas the MBM treatment demonstrated the lowest urease activity.

The beneficial effects of cover on final grain yield were dramatic and, on average, in treatments that did not receive anhydrous ammonia, exceeded 11%. Application of extra cover did not show a significant reduction in crown and root rot 64 DAP. At 140 DAP cover significantly reduced crown and root rot for the MBP compared to the MBM and the TP compared to the TM treatment. Differences between ANM and ANP and between FM and FP were not significant. Application of wheat straw cover did not significantly affect incidences of *F. graminearum* and *Diplodia/Stenocarpella* in crowns and roots. Earthworm counts were strongly correlated with infiltration rates and with soil moisture content. Treatments with extra wheat straw cover consistently had lower soil temperatures, higher soil moisture contents and higher earthworm populations. In addition, they had markedly reduced root lodging. It is particularly significant that even in maize fallow plots, where the quantity of maize residues approached 9 t/ha, extra wheat straw resulted in significant benefits in terms of soil temperature, soil moisture content, earthworm numbers, infiltration rate and root lodging. The grain yield differential between the FM and FP exceeded 1400 kg/ha. Comparison of these treatments in terms of the number of herbivorous, bacterivorous,

omnivorous and predator nematodes indicated that the effects 64 DAP were small. No meaningful trends in soil microbial activity and diversity were observed for the extra straw cover treatments 64 DAP. However, chemical biocides with cover (ANP and MBP) had higher numbers of different soil bacterial species, tillage and fallow with cover (TP and FP) had lower numbers of different soil bacterial species than their counterparts. BF and FM displayed a significant reduction in the number of different soil bacterial species between 64 and 140 DAP. Although not significant, added wheat straw cover resulted in higher enzyme activities and therefore a slightly higher potential to increase soil quality and fertility.

The results obtained this season again emphasised the effect of crown and root rot on grain yield with significant correlations between these parameters, as well as the complexity of soilborne diseases of maize and the effects of stress conditions on these diseases. It appears that different pathogens attack plants at different stages during the season and that these pathogens are affected by soil moisture and temperatures. Results of the first phase of the pathogenicity testing of fungi associated with diseased maize crowns and roots are reported in Facet 4 and have already provided valuable information that can be applied to research conducted for the field trial (Facet 1). However, more information is needed with regard to the possibility of winter crops as hosts of the most important soilborne diseases of maize. This, and the fact that treatments under field conditions need to be evaluated over many seasons to account for climatic differences clearly highlight the importance of long-term research to not only define all critical factors involved in soilborne diseases and the resultant yield losses in maize, but to implement strategies to significantly, sustainably and economically increase grain yield of maize in no-till systems.

Strategies such as sufficient straw cover to alleviate moisture stress and increase and earthworm numbers in soil, the latter being implicated to significantly reduce crown and root rot under the conditions of the field trial, and the application of anhydrous ammonia to increase Mn in plants, which also reduced crown and root rot, show great potential to significantly reduce soilborne disease problems and increase grain yield in maize. Measuring the effects of these practices on soil microbial activity and diversity, and earthworms is of paramount importance to ensure that these disease management strategies are sustainable and compatible with conservation farming practices.

In order to better understand the critical processes involved in no-till management and the effects of cover, further changes and additions will be made to the experimental protocol during the 2010/2011 season. The method of determining infiltration rate will be altered in order to reduce the possible influence of lateral water movement in

plots that have been rotovated or ripped. Permanent access tubes will allow measurement of subsoil moisture content at any stage during the season and employing 0-50-mm soil samples will provide critical information on the carbon and nutritional content of the soil immediately below the mulch layer. Due to financial constraints, not all the facets as proposed and described in the long-term proposal for this project will be conducted during the next season, but research will be prioritised to accomplish the proposed long-term objective to develop an integrated management strategy for no-till maize.

INTRODUCTION

No-Till, often referred to as “Zero-Till” or “Direct Seeding”, is considered to be one of the most revolutionary developments in agriculture in modern times (Bolliger *et al.*, 2006; Triplett & Dick, 2008). During the last three or four decades, its adoption by crop farmers in the Americas and elsewhere has been phenomenal. In Brazil, the area under no-till grew from less than 1000 ha in 1974 to over 22 000 000 ha in 2004 (Bolliger *et al.*, 2006) and in the USA, the area of cropped land under no-till currently exceeds 25 million hectares (Triplett & Dick, 2008). The area planted under no-till in Argentina is similarly huge and, as a percentage of cropped land, exceeds that in either the USA or Brazil (Roberto Pieretti, personal communication). In Canada and Australia, the combined area is in excess of 12 million hectares (Triplett & Dick, 2008). South Africa has been slow in adopting this specialised form of conservation agriculture, but Paterson (2007) indicated that the total area under no-till may be in the vicinity of 300 000 ha. This is perhaps questionable, but in KwaZulu-Natal, over 60 000 ha of maize and soyabean are annually planted without tillage and adoption of the practice is growing rapidly.

Initially, the primary reasons for switching to no-till were to reduce soil and wind erosion, increase infiltration and water-use efficiency and reduce both the energy and power requirements of cropping. In other words, to conserve and rehabilitate the soil and environment and to reduce production costs. Results in this respect have been spectacular and today, most no-till fields in KwaZulu-Natal and elsewhere do not rely on contours to control runoff and soil loss. It seems that the 30–40% soil cover commonly provided by untilled fallows of crops such as maize, is adequate for this purpose.

More recently, however, it has been recognised that in many soil-bioclimate systems, particularly where moisture is the primary long-term yield-limiting factor, as is the

case in South Africa, that the soil cover provided by a simple off-season fallow may be appreciably less than optimal with regard to performance of the primary crop. This is particularly true for soyabean, which itself produces poor cover. Consequently, an increasing amount of research is being conducted into the possible role of cover crops; crops planted in the off-season in order to elevate biomass loads for the main crop (Villamil *et al.*, 2006; Calegari *et al.*, 2008; Singer, 2008).

Other effects of no-till agriculture, which are currently attracting considerable attention internationally, include the effects on carbon sequestration, global warming and, of course, the potential for cellulosic biofuel production. These issues, however, are “peripheral” to our study, even though the research vehicle (field experiment) may eventually provide critical information in this regard.

The primary aim of this study is to determine the role and importance of soilborne diseases and microbial diversity in no-till maize double cropped with winter wheat. Ultimately, it is hoped that remedial intervention strategies will be developed for this management system and also for dryland continuous maize, the dominant management system in South Africa.

Initially, it was anticipated that crop rotations employing crops other than wheat, the use of biocontrol agents, chemical biocides, seed treatment, and perhaps even tillage, might provide solutions (Lamprecht *et al.*, 2007). However, in the first season of experimentation this did not prove to be the case and, with the exception of anhydrous ammonia application as a source of nitrogen, none of the alternative strategies proved successful. Surprisingly, the control treatment, maize following winter wheat, had high root and crown rot severity indices, performed poorly up to 100 days after planting, but in terms of final grain yield, significantly out-yielded treatments with less biomass cover – even the maize fallow with approximately 10 t/ha of maize stover and the methyl bromide fumigated treatments. Early season yield benefits to all treatments other than anhydrous ammonia application had apparently been overwhelmed by the benefits of cover during a prolonged period of moisture stress in February. This led to a radical change in the trial design and treatments, which would facilitate a direct comparison between plots with and without extra cover were incorporated (Lamprecht *et al.*, 2008).

In the unusually favourable 2007/2008 season – average yield was almost 2900 kg/ha higher than that in the previous season – this decision was partially justified. On average, extra cover resulted in a small, but non-significant yield benefit, but the control treatment was the poorest performer in the trial and was out-yielded highly

significantly by alternative rotations, anhydrous ammonia use, methyl bromide fumigation, tillage, and some of the biocontrol products. Similar effects were evident in terms of root and crown rot severity and we speculated, that absence of moisture stress had negated the positive effects previously brought about by cover (Lamprecht *et al.*, 2008).

This led to further changes in the experimental protocol during the 2008/2009 season, in order to better understand what effects cover was having on soil properties. Earthworm counts, a widely accepted index of soil macroporosity, were conducted and so, too, were topsoil (0–60 mm), temperature and moisture determinations. In addition, a bare fallow treatment, in which all maize residues were removed after harvest, was introduced in order to more accurately assess the benefits provided by conventional maize fallows, and stalling rye, another high biomass producing winter crop, replaced a poorly performing biocontrol product. These modifications to the trial proved valuable. Late-season moisture stress occurred during March and dramatic cover effects were visually evident. On average, the benefits of plus- relative to minus-cover analogues amounted to over 1200 kg/ha of grain, topsoil moisture content was significantly higher, temperatures were consistently lower, earthworm counts were higher, and crown and root rot severity ratings were lower. Significantly, in all these respects the bare fallow treatment was appreciably worse than any other treatment in the trial (Lamprecht *et al.*, 2009).

Results obtained over the last three seasons are, thus, complicated by the fact that, although in each season the control treatment (maize after winter wheat) has had among the worst root and crown rot severity ratings in the trial, in two seasons in which moisture stress was experienced it was only significantly out-yielded by the anhydrous ammonia treatment. Only in the exceptionally favourable 2007/2008 season did it perform worse than the other treatments in the trial. This suggests that in the presence of moisture stress, the disadvantages associated with diseased roots are being compensated for by the benefits of cover. In a sense, this is illogical, as it would be reasonable to assume that plants with already damaged root systems would suffer most when moisture was the primary limiting factor. At present this cannot be adequately explained, but the possibility exists that earthworm-created macroporosity may be involved. In the 2008/2009 season, earthworm activity in control plots was significantly higher than that in any other treatment 127 days after planting (DAP) (Lamprecht *et al.*, 2009). This could have increased the soil's infiltration rate and enhanced subsoil moisture accumulation.

In an effort to obtain more information in this regard, the precision of earthworm counts has been increased this season by doubling the number of traps per plot and infiltration rate determinations have been initiated. Such information, it is hoped, will also help to separate the effects of enhanced infiltration from those of reduced water loss by evaporation from the soil surface. The literature in this regard is somewhat vague and more emphasis is placed on infiltration effects than on the insulating effects of cover on soil properties near the surface. Some authorities believe that the latter are of little consequence once the crop has canopied (Triplett & Dick, 2008), but it is at this stage of development that minus-cover plots have fallen behind their plus-cover analogues during two out of three seasons of this investigation.

OBJECTIVES

The overall objective of this investigation is to establish practical and economic ways of reducing the yield-limiting effects of soilborne diseases in no-till systems of maize production. Specific objectives of this facet are to (1) assess the effects of pathogenic fungi and parasitic nematodes on the growth of maize following winter wheat, (2) identify and quantify the species involved, (3) examine the effects of alternative winter rotational crops, (4) measure the ameliorative effects of biological control products marketed to farmers and of potential chemical biocides, (5) compare soil disturbance after winter wheat in the place of no-till, (6) establish the importance and role of surface cover in disease suppression and yield, and (7) determine the influence of these potentially ameliorative strategies on microbial activity and diversity in the soil. Methyl bromide fumigation is employed to provide the targeted baseline to be simulated, not as a practical or economic alternative.

MATERIALS AND METHODS

The trial is located in the Winterton district on the farm of Mr Anthony Muirhead. The soil is a Hutton sandy clay, a soil type similar to many other maize producing soils in South Africa. Selected physical and chemical properties of a representative profile are presented in Table 1. The site has been under no-till for many years, but is outside the pivot circle and prior to 2006 had not previously been cropped during the winter. In order to grow the winter crops needed to test the rotational effects of wheat, canola, crambe, black oat and stooling rye, irrigation is provided with a movable system operated by the farmer.

Throughout this investigation, supplementary irrigation has also been available for the primary maize phase of the trial. However, its use has been restricted to potentially critical periods and in one season (2008/2009) was not used at all.

The winter program was initiated immediately after maize from the 2008/2009 season had been harvested. At the same time maize residues were removed from bare fallow (BF) plots and, in order to reduce the possible effects of K stratification, all plots received a surface application of 100 kg K/ha. All except the fallow plots also received 80 kg N/ha a month after establishment.

On 5 October 2009 the various winter crops (wheat, canola, crambe, black oat and stouling rye) were killed with glyphosate. Approximately one month later, wheat straw was cut and removed from all plots to receive anhydrous ammonia (ANM, ANP & ANM+OB), those to be similarly ripped, but without gas application (CRI), plots to be rotovated prior to methyl bromide fumigation (MBM & MBP) and those to be rotovated, but not fumigated (TM & TP). Straw removed was stacked adjacent to individual plots for later use where required in plus-cover treatments (ANP, MBP, TP & FP). Plots to be methyl bromide fumigated and their unfumigated analogues were then thoroughly rotovated and methyl bromide was applied at a rate of 1000 kg/ha on 13 November 2009. Plots were left covered and sealed for 48 hours to ensure optimal penetration of the gas.

Anhydrous ammonia was applied to relevant plots at a rate of 200 kg N/ha on 27 November 2009. On the same day the control-rip (CRI) plots were established with the equipment used to apply anhydrous ammonia.

The experiment was planted on 30 November 2009 to the maize cultivar PHI 32D96B at a population of approximately 53 000 plants per ha. Three hundred kg/ha of boronated superphosphate was applied in the row. The first application of biocontrol products (Eco-T, Fungimax plus Organoboost, and Gliogrow) occurred immediately after planting as a 150-mm wide drench over the plant row.

Three DAP, LAN was applied to all except the anhydrous ammonia plots at a rate of 200 kg N/ha and yield assessments of the various cover crops were conducted using a randomly positioned 0.25 m² steel ring. Oven dry mass determinations indicated that the winter covers had provided 9440, 8760, 7040, 3920 and 2840 kg/ha in the case of black oat, stouling rye, wheat, canola and crambe, respectively. The oven dry mass of residual maize residues in fallow plots was 9760 kg/ha. Thus, at the start of the season, total biomass cover ranged from zero in bare fallow plots, through about

9700 kg/ha in maize fallow plots, to over 19000 kg/ha in plots that had been planted to black oat.

Eighteen DAP, wheat straw was hand-applied inter-row to plus-cover plots. At the same time, 50 kg/ha of K was applied to minus-cover plots to compensate for K being returned in the form of straw to plus-cover analogues. The second application of biocontrol products also took place at this time.

Ten days later (28 DAP), the first plant sampling was done. Six whole plants were cut and removed from each plot, oven dried, weighed and subsequently milled and chemically analysed at Cedara. At this time, inter-row soil samples (0-150 mm) were also collected for chemical analysis at Cedara, 25 subsamples being combined from each plot.

The first soil temperature and moisture determinations (0-60 mm) were conducted 42 DAP and shortly thereafter (46 DAP) two earthworm traps (0.25 m² steel confinement rings) were embedded in each plot.

The first earthworm counts were conducted 60 DAP, using 20 L of 0.4% formalin solution to drive worms to the surface in the steel confinement rings. Sixty-four DAP the first soil and plant sampling for soilborne disease and microbial diversity and activity assessment occurred. Eight plants were removed from each plot, above-ground plant parts were weighed and roots were washed. Subsamples of roots were collected for nematode analysis and the remainder of the samples taken to Stellenbosch for crown and root rot severity ratings and fungal identification and incidence assessment. At the same time, rhizosphere soil samples were collected for nematode analyses and microbial activity and diversity determinations in Pretoria. Seven days later (at 50% silking), leaf samples (15 index leaves per plot) were collected for chemical analysis at Cedara.

The second soil temperature and moisture determinations were conducted 73 DAP and the earthworm traps were repositioned 121 DAP.

The third soil temperature and moisture readings and the second earthworm counts were conducted 135 DAP. Five days later, the second sampling for soilborne disease assessment, microbial analysis, and above-ground yield determination was conducted. Sampling was as described previously, but no samples were collected for nematode analysis. However, rhizosphere soil samples were also collected for analysis by Omnia Fertilizer using the Omnibio procedures. The following day, the number of

root-lodged plants per plot was determined, as the prevalence of lodged plants was very clearly treatment related.

In an effort to better understand the effects of cover on plant growth, and soilborne diseases, infiltration-rate comparisons were conducted 154 DAP by determining the time required for 20 L of water to infiltrate into one set of the 0.25 m² confinement rings used as earthworm traps. Since only relative differences between treatments were considered necessary for exploratory work, no attempt was made to separate the effects of lateral and vertical subsoil water flow. However, in the 2010/2011 season, in an effort to refine these measurements, it is the intention to use both earthworm traps in each plot and to perform the infiltration tests the day after earthworm counts are done. This should appreciably reduce the influence of lateral water flow and improve the precision of measurements obtained.

Table 1. Selected physical and chemical properties of a representative soil profile.

Depth	Sample Density	P	K	Ca	Mg	Zn	Mn	pH (KCl)	Exch. Acidity	Acid Sat.	Total C
mm	g/mL	mg/L							cmol/L	%	%
0-150	1.11	104	89	1056	210	10.0	4	4.73	0.13	2	1.25
150-300	1.14	18	84	482	113	5.0	8	4.02	1.18	25	0.93
300-450	1.14	5	61	574	134	2.3	4	4.34	0.39	9	0.86
450-600	1.15	6	49	647	126	0.9	1	4.84	0.11	2	0.69
600-750	1.13	2	48	569	100	0.9	1	4.73	0.12	3	0.49
750-900	1.15	4	56	582	129	0.9	1	4.55	0.18	4	0.38

Treatments created during the previous winter season or imposed shortly before or after planting during the 2009/2010 season are shown in Table 2. This season, the nematicide, Crop Guard, having consistently proved ineffective during the previous three seasons, was replaced by Gliogrow, a recently released fungicide. Points presented below Table 2 provide further rationale for selection of the 19 treatments. Plot dimensions were 7.25 x 9.5 m and there were eight rows per plot (two border rows and six net rows). All plots were separated transversely by a 1-m pathway and there was an additional border area of 1 m at both ends of each plot.

Table 2. Treatments included in the field trial.

Treatment Code	Treatment	Previous Summer Crop	Winter Crop	Current Crop
ANM	Anhydrous ammonia minus extra wheat straw	Maize	Wheat	Maize
ANP	Anhydrous ammonia plus extra wheat straw	Maize	Wheat	Maize
ANM+OB	ANM plus Fungimax and Organoboost	Maize	Wheat	Maize
BF	Fallow with all maize residue removed	Maize		Maize
BO	Black oat	Maize	Black oat	Maize
C	Control	Maize	Wheat	Maize
CAN	Canola	Maize	Canola	Maize
CR	Crambe	Maize	Crambe	Maize
CRI	Control ripped	Maize	Wheat	Maize
ECO	Eco-T	Maize	Wheat	Maize
FM	Maize fallow minus extra wheat straw	Maize		Maize
FP	Maize fallow plus extra wheat straw	Maize		Maize
GLIO	Gliogrow	Maize	Wheat	Maize
MBM	Methyl bromide minus extra wheat straw	Maize	Wheat	Maize
MBP	Methyl bromide plus extra wheat straw	Maize	Wheat	Maize
OB	Fungimax + Organoboost	Maize	Wheat	Maize
SR	Stooling Rye	Maize	Rye	Maize
TM	Tilled minus extra wheat straw	Maize	Wheat	Maize
TP	Tilled plus extra wheat straw	Maize	Wheat	Maize

The rationale for inclusion of these treatments is as follows⁴:

ANM	—	Anhydrous ammonia is a popular N source and there is evidence that it has biocidal properties.
ANP	—	
ANM+OB	—	OB (Fungimax + Organoboost) is a fungal and bacterial feedstock, which might ameliorate the sterilizing effects of anhydrous ammonia.
BF	—	A fallow with maize residues removed after harvest so as to better measure the influence of the FM treatment.
BO	—	Black oat is considered in Brazil to be an excellent rotational crop and source of cover.
C	—	Wheat-maize rotation against which to measure treatment responses.
CAN	—	Canola is a possible alternative winter crop to wheat.
CR	—	Crambe is another alternative winter crop to wheat.
CRI	—	Ripped control treatment to separate the effects of anhydrous ammonia <i>per se</i> from those of the ripping action associated with its application.
ECO	—	Eco-T is a currently registered and marketed biocontrol agent.
FM	—	Winter maize fallow to determine whether omitting wheat provides any benefit to following maize. Maize fallows are also dominant in South African reduced tillage systems.
FP	—	
GLIO	—	Gliogrow is a recently released and registered biocontrol agent.
MBM	—	Methyl bromide temporarily eliminates soilborne diseases and is used here to provide a baseline of the potential maize yields attainable.
MBP	—	
OB	—	Fungimax + Organoboost is currently marketed to enhance fungal and bacterial populations.
SR	—	Stooling rye is a promising alternative winter crop. It is tolerant to drought and acidity and is resistant to decomposition.
TM	—	Tilled without MB to separate the effects of methyl bromide <i>per se</i> from those of the tillage associated with its application.
TP	—	

♣ ANM, FM, MBM and TM are treatments without extra wheat straw, while ANP, FP, MBP and TP are analogues to which 7 t/ha of wheat straw was added 18 DAP in order to measure the effects of extra cover.

This season, because of the need to substantially reduce costs, the experiment was again only sampled twice for disease assessments and microbial analyses (64 & 140 DAP).

Prior to being flown to Stellenbosch for root rot assessments and fungal identification, roots were washed in distilled water to remove excess soil and were stored in cooler boxes in a cold room. Roots from all plants sampled 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.

Isolations were done from all plants collected. Plant roots were washed under 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. 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 (20 root and 20 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.

The nematode samples were collected during the first week of February 64 DAP. Each sample was placed in a labelled plastic bag, transported to the laboratory and stored at 12° C. Each sample was thoroughly mixed before a 250 cm³ soil sub-sample was taken from it. Nematodes were extracted according to the sieving-centrifugation-flotation method (Kleynhans, 1997). The roots were rinsed free from soil and cut in smaller pieces (\pm 20 mm), the material (20 g) was shredded in a food blender, washed through 1000 μ m, 150 μ m, 45 μ m and 38 μ m aperture sieves. The residue of the last three sieves was transferred to 100 ml centrifuge tubes and the nematodes extracted according to the centrifugation-flotation method. The population numbers of the plant-parasitic nematodes (herbivores) were determined by withdrawing a sub-sample into a De Grisse counting dish, identifying the nematodes to genus level and counting the number of specimens of each genus with a Laboratory DC Counter. For quick identification to species level, the nematodes were mounted on temporary slides and identified with a research microscope by the relevant experts in the Nematology Unit (ARC-PPRI). The population numbers of the other groups of free-living nematodes as defined by Yeates *et al.* (1993) were also determined using the same method and during the same time as that of the plant-parasitic component.

Soil samples for analysis of functional diversity of microbial populations were stored at $\pm 5^{\circ}$ C prior to analysis, and samples for enzyme activities were dried at 40° C for 48 h, sieved (< 2 mm) and also stored at $\pm 5^{\circ}$ C. Soil samples were diluted (1:3,000) and plated onto Biolog EcoPlatesTM (Biolog[®] Inc., Hayward, USA) containing 31 carbon sources and a control well, in triplicate. The plates were incubated at 25° C and optical density at 590 nm was measured twice daily over a period of 5 days. The functional diversity of the soil microbial population was determined using the amount and equitability of carbon substrates metabolized as indicators of richness and evenness, respectively.

In order to determine the effect of the different treatments on the ability of the soil population to obtain carbon, phosphorus and nitrogen, the β -Glucosidase, alkaline phosphatase, acid phosphatase, and urease activities in the soil samples were assayed. β -Glucosidase and phosphatase activities were calculated using the release of *p*-nitrophenyl after the incubation of soil with *p*-nitrophenyl glucoside and *p*-nitrophenyl phosphate, respectively. Urease activity was calculated from the ammonia released after incubation of soil with a urea solution.

STATISTICAL ANALYSES

The experimental layout used in the study was a randomised complete block design consisting of 19 treatments in three replications. All acquired data were statistically analysed by the ARC statistician in Stellenbosch.

Data on carbon utilisation were subjected to non-parametric statistical analyses using STATISTICA 6 (StatSoft, Inc ©). Carbon substrate utilisation profiles and enzyme activity were statistically analysed by principal component analysis (PCA) (Palojärvi *et al.*, 1997) and homogenous grouping with Fisher Least Significant Difference (LSD). Biodiversity was determined using the Shannon-Weaver diversity index and substrate evenness index, which indicates species richness and the variation between species within the local soil microbial community, respectively (Magurran, 1988).

RESULTS AND DISCUSSION

General information on parameters measured

The season was below average in terms of precipitation and was unusually cool during the early part. Severe moisture stress was evident 30 DAP and again during February (Table 3). March precipitation was above average, but by this stage the plants were approaching physiological maturity.

As is evident in Table 3, seasonal variability has been appreciable over the period of investigation and it appears that post-flowering moisture stress has played a critical role with respect to the treatment responses to be discussed.

Table 3. Rainfall recorded during the period of investigation (supplementary irrigation shown in brackets).

October	November	December	January	February	March	Total
mm						
2006/2007						
82	157 (10)	148 (10)	131	30	102	670
2007/2008						
124	65 (10)	110 (10)	194 (50)	105	124	792
2008/2009						
30	112	152	176	148	49	667
2009/2010						
78	52	109 (50)	126	47	142	604

In the interests of clarity, it is the intention here to separately discuss the effects of groups of similar treatments. Rotations using canola (CAN), crambe (CR), black oat (BO), stouling rye (SR), fallow without extra wheat straw cover (FM) and bare fallow (BF) will form one group, the biocontrol agents OB, ECO, GLIO and ANM+OB another, the chemical biocides MBM and ANM a third, tillage (TM and CRI) a fourth and the effects of added wheat straw cover (FP, MBP, ANP and TP) the final group.

The main findings in each section are presented in a summarized format in the "Summary and Conclusions" section at the end of this report.

Crown and root rot severity

Crown and root rot severity were significantly positively correlated 64 DAP ($P < 0.0001$, $r = 0.936$) and also 140 DAP ($P < 0.0001$, $r = 0.967$). There were also significant negative correlations between crown ($P = 0.0008$, $r = -0.706$) and root rot ($P = 0.0012$, $r = -0.687$) severity 140 DAP and grain yield. This confirms results obtained during the previous three seasons. At 64 DAP, crown ($P = 0.0211$, $r = -0.524$) and root rot ($P = 0.0266$, $r = -0.507$) severity were significantly negatively correlated with Mn levels in plants, and Mn levels in plants were significantly positively correlated with grain yield ($P = 0.0067$, $r = 0.599$).

Fungi associated with crowns and roots

Fungi isolated from crowns and roots in this study were similar to those recorded during previous seasons. Fungi most frequently isolated from crowns and roots were *Acremonium* spp., *Diplodia/Stenocarpella* spp., *F. equiseti*, *F. graminearum*, *F. oxysporum*, *F. proliferatum*, *F. solani*, *F. subglutinans*, *Phialophora* spp., *Pyrenochaeta terrestris* and *Trichoderma* spp. Fungi infrequently isolated are listed in Table 4, and were not subjected to statistical analysis. *Pythium* spp. were infrequently obtained, but we included these fungi in the analyses in order to compare their occurrence with results obtained during the previous seasons. Treatments and sampling time effects on the incidences of the fungi in crowns and roots are listed in Table 5. All these fungi were isolated from crowns and roots and as in previous seasons, *Acremonium* spp., *Diplodia/Stenocarpella* spp., *F. proliferatum* and *F. subglutinans* were obtained more frequently from crowns than roots. The effects of sampling time on the incidences of fungi are listed in Table 6. *Fusarium equiseti*, *F. oxysporum*, *F. proliferatum*, *F. solani*, *P. terrestris*, *Pythium* spp. and *Trichoderma* spp. were not affected by sampling time, whereas *Acremonium* spp., *Diplodia/Stenocarpella* spp., *F. subglutinans* and *Phialophora* spp., significantly increased from the first to the second sampling time. *Phialophora* spp. in roots 64 DAP were significantly negatively correlated with grain yield ($P = 0.0161$, $r = -0.543$) and soil moisture 135 DAP ($P = 0.0385$, $r = -0.477$). Incidences of *Diplodia/Stenocarpella* spp. in roots 140 DAP were significantly negatively correlated with grain yield ($P = 0.485$, $r = -0.458$). Incidences of *Diplodia/Stenocarpella* spp. and *F. graminearum* in roots were also significantly negatively correlated ($P = 0.0253$, $r = -0.511$) 64 DAP. *Fusarium graminearum* in roots 64 DAP was also significantly positively correlated with soil moisture ($P = 0.442$, $r = 0.544$) and incidences in crowns 140 DAP were significantly positively correlated ($P = 0.0173$, $r = 0.538$) with lodging. Significant

sampling time x treatment interactions were recorded for *F. graminearum* (crown) and *Phialophora* spp. (crown) (Table 5). All the fungi frequently isolated have previously been recorded as pathogens of maize (Sumner & Bell, 1982; Ramsey 1990).

Similar to previous seasons, *F. oxysporum*, followed by *Trichoderma* spp., *F. graminearum* and *P. terrestris* were the fungi most frequently isolated from crowns and roots of maize plants in this trial (Lamprecht *et al.*, 2009). An overview of their significance as pathogens of maize, as well as the significance of other isolated fungi such as *Acremonium* spp., *Diplodia/Stenocarpella* spp, *F. equiseti*, *F. solani*, *F. proliferatum*, *Phialophora* spp and *Pythium* spp., as published by previous researchers in South Africa and other countries has been given in detail in previous reports and will not be repeated here.

Table 4. Fungi infrequently isolated from crowns and roots collected from the different treatments.

Fungus	Crowns ^z	Roots ^z
<i>Alternaria</i> spp.	+	+
<i>Apergillus</i> spp.	+	+
<i>Bipolaris</i> spp.	-	+
<i>Chaetomium</i> spp.	+	+
<i>Cladosporium cladosporioides</i>	+	+
<i>Epicoccum</i> spp.	+	+
<i>Fusarium nygamai</i>	+	+
<i>Fusarium scirpi</i>	-	+
<i>Fusarium semitectum</i>	+	+
<i>Fusarium</i> spp.	+	+
<i>Fusarium verticillioides</i>	+	+
<i>Gliocladium roseum</i>	+	+
<i>Macrophomina phaseolina</i>	+	+
<i>Mortierella</i> spp.	+	+
<i>Neocosmospora vasinfecta</i>	-	+
<i>Penicillium</i> spp.	+	+
<i>Phoma</i> spp.	+	+
<i>Pythium</i> spp.	+	+
<i>Rhizoctonia</i> spp.	+	+
<i>Rhizopus</i> spp.	-	+
Sterile fungi	+	+
Unidentified fungi	+	+

^z + = Fungus isolated; - = Fungus not isolated.

Table 5. Significance levels (P values) of the effect of treatment and sampling time on the incidence of fungi in crowns and roots.

Factors	Plant part	Fungi ^y												
		Acrem	Diplo/Steno	Fequi	Fgram	Foxy	Fprol	Fsola	Fsubg	Phia	Pyren	Pyth	Trich	
Treatment	Crown	0.0081 ^z	NS	NS	0.0017	NS	NS	NS	NS	NS	NS	NS	NS	0.0007
	Root	NS	NS	NS	<0.0001	NS	NS	NS	NS	NS	NS	NS	0.0026	NS
Sampling Time (S)	Crown	0.0065	0.0331	NS	0.0009	NS	NS	NS	NS	NS	0.0003	0.0405	NS	NS
	Root	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.0120	0.0002	NS	NS
T x S	Crown	NS	NS	NS	0.0033	NS	NS	NS	NS	NS	NS	0.0118	NS	NS
	Root	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

^y Acrem = *Acremonium* spp., Diplo/Steno = *Diplodia/Stenocarpella* spp., Fequi = *Fusarium equiseti*, Fgram = *Fusarium graminearum*, Foxy = *Fusarium oxysporum*, Fprol = *Fusarium proliferatum*, Fsola = *Fusarium solani*, Fsubg = *Fusarium subglutinans*, Phia = *Phialophora* spp., Pyren = *Pyrenochaeta terrestris*, Pyth = *Pythium* spp., Trich = *Trichoderma* spp.

^z NS = not significant.

Table 6. 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	
<i>Acremonium</i> spp.	Crown	1.2b	2.8a	1.10
	Root	0.3a	0.1a	NS
<i>Diplodia/Stenocarpella</i> spp.	Crown	0.2b	1.5a	1.20
	Root	0.3a	0.4a	NS
<i>F. equiseti</i>	Crown	0.0a	0.3a	NS
	Root	0.7a	1.3a	NS
<i>F. graminearum</i>	Crown	Inter.	Inter.	Inter.
	Root	13.0a	15.9a	NS
<i>F. oxysporum</i>	Crown	5.9a	6.8a	NS
	Root	44.9a	39.8a	NS
<i>F. proliferatum</i>	Crown	1.5a	1.3a	NS
	Root	0.5a	1.1a	NS
<i>F. solani</i>	Crown	0.1a	0.7a	NS
	Root	4.2a	4.1a	NS
<i>F. subglutinans</i>	Crown	6.1b	12.7a	3.31
	Root	3.3b	6.5a	2.42
<i>Phialophora</i> spp.	Crown	Inter.	Inter.	Inter.
	Root	0.4b	2.5a	1.02
<i>Pyrenochaeta terrestris</i>	Crown	1.1a	1.6a	NS
	Root	6.6a	8.0a	NS
<i>Pythium</i> spp.	Crown	0.0a	0.2a	NS
	Root	0.5a	0.7a	NS
<i>Trichoderma</i> spp.	Crown	4.0a	4.0a	NS
	Root	26.6a	22.6a	NS

^wST = Sampling time; ST1 = 64 DAP, ST2 = 140 DAP.

^xMeans within a fungus, within a plant part followed by the same letter do not differ significantly (P = 0.05).

^yInter. = Indicates a significant treatment x sampling time interaction; See Table 18 for interaction means.

^zLSD = Least significant difference at P = 0.05; NS = Not significant.

Nematodes

As an introduction to the nematode results it is necessary to mention some of the general findings. The term herbivore for plant-parasitic or plant feeding nematodes will be used throughout the report.

The lesion nematodes, *Pratylenchus brachyurus* and *Pratylenchus zae* were the most common endoparasites. *P. zae* was the predominant lesion nematode species found at 94% of the sites. Root-knot nematodes (*Meloidogyne* spp.) were the only other endoparasites found. *Scutellonema brachyurus*, *Paratrichodorus minor* and *Longidorus pisi* were the most common ectoparasites and found at 70%, 49% and 31% of the sites, respectively. The semi-endoparasitic nematodes *Helicotylenchus dihystera* and *Rotylenchulus parvus* were found at 83% and 86% of the localities, respectively (Table 7). The effect of nematodes on maize in general and more specifically the nematodes found at the trial site were discussed in Lamprecht *et al.* (2007, 2008, 2009). *Scutellonema unum*, found for the first time during the current season, was described from pineapple in Kenya and recorded around citrus, *Eucalyptus* and veld in the Kwazulu-Natal, Mpumalanga, North West, and Western Cape provinces (Sher, 1963; Kleynhans *et al.*, 1996). The first report from maize in South Africa was from the Limpopo Province (Marais & Swart, 2002).

Conventionally, all nematodes that are not animal parasites are called free-living, including the herbivores or plant parasitic nematodes (Poinar, 1983). However, in this discussion the term free-living will be used in a more restricted sense to refer only to non-plant-parasitic terrestrial and freshwater nematodes. Free-living nematodes or beneficial nematodes are an integral part of the interlocking chain of nutrient conversions. They function in the recycling of carbon-containing substances, mineral nutrients and nitrogenous components. Likewise they control explosions of microflora and microfauna and maintain the balance of life forms that constitute the delicate balance of nature. Viglierchio (1991) duly emphasized the fact that although free-living nematodes are considered benign by mankind, they constitute one of the vital components in the preservation of the balance of life processes of our world.

Nematodes that are normally encountered in freshwater habitats (e.g. streams, lakes, dams, karst systems) are all microscopic in size, ranging from about 0.25 to 10 mm in length. Although some of these may be several mm long, they are seldom more than 30 to 40 μm in diameter, so that they are normally not visible to the eye (Heyns, 2002). These nematodes may enter cultivated soil by means of irrigation water or flooding.

Table 7. Plant-parasitic nematodes found in the soil and roots of the different treatments.

Nematode species	Common Name	Incidence	
		In roots ^a	In soil ^a
1. Belonolaimidae			
<i>Tylenchorhynchus brevilineatus</i> Williams, 1960	Stunt nematodes	0	33
2. Pratylenchidae			
<i>Pratylenchus brachyurus</i> (Godfrey, 1929) Filip'ev & Schuurmans Stekhoven, 1941	Lesion nematode	46	9
<i>Pratylenchus zaeae</i> Graham, 1951	Lesion nematode	89	98
3. Hoplolaimidae			
<i>Helicotylenchus dihystra</i> (Cobb, 1893) Sher, 1961	Spiral nematode	68	98
<i>Scutellonema brachyurus</i> (Steiner, 1938) Andr�ssy, 1958	Spiral nematode	40	98
<i>Scutellonema unum</i> Sher, 1963	Spiral nematode	4	4
<i>Rotylenchulus parvus</i> (Williams, 1960) Sher, 1961	-	84	88
4. Heteroderidae			
<i>Meloidogyne</i> sp. (Immature)	Root-knot nematodes	2	2
5. Criconematidae			
<i>Criconemoides sphaerocephalus</i> Taylor, 1936	Ring nematode	2	33
6. Trichodoridae			
<i>Paratrichodorus minor</i> (Colbran, 1956) Siddiqi, 1974	Stubby-root nematode	0	98
7. Longidoridae			
<i>Longidorus pisi</i> Edward, Misra & Singh, 1964	Needle nematode	0	61
8. Belondiridae			
<i>Dorylaimellus</i> sp. (unidentified)	-	0	11

^a Incidence in total number of samples collected 64 DAP.

Free-living nematodes are common in natural veld and some species are particularly common in cultivated fields in South Africa. These include members of the Rhabditida, Cephalobida, Panagrolaimida, Tylenchida, Dorylaimida, Enoplida, Aerolaimida, Triplonchida and Mononchida (Heyns, 1971). For the agriculturalist, free-living soil-inhabiting nematodes, or soil nematodes for short, are of particular interest. They are small, generally between 0.3 – 5.0 mm long and can be abundant (in their millions) but also diverse (commonly more than 30 taxa) in all soils (Yeates, 1979). As nematodes feed on a wide variety of soil organisms and are

dependent on the continuity of soil water films for movement, their activities are largely controlled by the biological and physical conditions of the soil. According to Van Bruggen & Semenov (in Pattison *et al.*, 2006), a healthy soil is resilient and able to recover from stress, has a high biological diversity and high levels of nutrient recycling. Plant feeding organisms impose biological stress on plants and therefore, a healthy soil should have mechanisms that enable it to regulate the proportion of parasitic organisms. Soil conditions, therefore, need to be manipulated by farm management practices to enhance the natural suppression that exists in soil systems and regulate, for instance, herbivores (Pattison *et al.*, 2006). One method of manipulating these conditions is through the use of amendments. Pattison *et al.* (2006) recommended paired surveys comparing soil under cultivation to soil from less intensively managed vegetation systems to develop key soil health indicators. Such indicators are soil C, $\text{NO}_3\text{-N}$, pH, nematode diversity and incidence of herbivores. As an example, a study of soil quality improvement and nematode management on banana farms in Australia (Pattison *et al.*, 2006) showed, among other things, that management practices that increased the amount of labile C, decreased $\text{NO}_3\text{-N}$, increased the level of diversity of soil organisms, and also increased the suppression of plant parasitic nematodes in banana crops, making banana production more sustainable.

Although the body form of soil nematodes is basically the same in all stages, their greatest apparent morphological diversity can be seen in the head and mouth structures, which are closely related to their feeding habits. In many studies on the relationship between nematode community structure and various agricultural practices, the trophic groups are reduced to five main groups: bacterivores, fungivores, herbivores (plant parasitic nematodes), omnivores and predators. Given this range of feeding types, the soil nematode fauna interact with many other groups of soil organisms and, therefore, play a critical role in controlling the mineralization of nutrients for plant growth. In soil, nematodes, collembolans and mites are three groups of mesofauna considered as important biological indicators. Of the three groups, nematodes may be the most suitable for environmental diagnoses based on the community structure analysis, especially as more information exists on their taxonomy and feeding roles (Gupta & Yeates, 1997) than for other mesofauna.

Organisms in the soil tend to be aggregated in areas where carbon and energy originally enters the food web, e.g., the plant rhizosphere, close to the soil surface or in the tillage zone. Under cropping and horticultural regimes there is regular disturbance of soil, soil fauna and plant cover. The decrease in diversity of nematodes with increased level of management reflects not only physical disturbance and change

in the quality and quantity of organic matter being returned to the soil, but also possible increases in specific herbivorous nematodes associated with crops.

The decrease in diversity of nematode fauna with increasing levels of management reflects not only physical disturbance and change in quality of organic matter being returned to the soil, but also possible increases in specific herbivorous nematodes associated with crops (Yeates & Bongers, 1999). Generally, soils with annual arable crops contain fewer nematode species, whereas up to 154 species have been recorded in grasslands (Hodda & Wanless, 1994). Burial of plant material during cultivation provides resources for microbial-feeding nematodes whose populations may exceed 3000 g⁻¹ dry matter (Sohlenius & Boström, 1984). Initially the populations tend to be dominated by bacterivorous genera of the Rhabditidae, Cephalobidae and Panagrolaimida with fungivorous Aphelenchidae contributing during later stages of decomposition. Studies of the rhizosphere of peas, barley, turnips and grass showed that the biomass of microbial-feeding nematodes exceeded that of protozoa (Griffiths, 1990) and led to the conclusion that nematodes are more important than protozoa in terms of nutrient cycling. In a Dutch study (Bouwman & Zwart, 1994) arable fields under integrated management receiving lower agro-chemical and tillage inputs consistently had increased total nematode biomass. The greatest increase was in herbivores, but omnivores/predators were also always greater under integrated management. While the numbers of fungivores were lower, bacterivores comprised the dominant feeding group at 40–69%. Hendrix (1999) showed that no-tillage management favors foodwebs dominated by fungi and fungivores and high numbers of earthworms. In contrast, foodwebs in ploughed soils show greater importance of bacteria and bacterivores, which colonize buried residues. As a consequence of these altered biotic communities, residue decomposition, organic matter mineralization and nutrient release rates tend to be higher in ploughed than in no-till soils.

To compensate for the effect of the feeding behaviour of the different herbivorous nematodes, the results of the nematodes extracted from the roots and soil were combined and are given in Table 8, Fig. 1, and Fig. 2. In the discussion of treatment effects on the incidence of beneficial nematodes (bacterivores, fungivores, omnivores and predators) against that of plant parasitic nematodes (herbivores), the following were taken into account:

- The population numbers of omnivores and predators were so low in all treatments, that their role in the ecological soil processes is deemed to be negligible in the present study. Mention of beneficial nematodes in the discussion that follows will therefore include only the bacterivores and

fungivores and exclude the omnivores and predators. The low population numbers of the omnivores and predators are typical of regular disturbances of soil, soil fauna and plant cover in an agricultural regime. Freckman and Ettema (1993) found that the relatively large predators and omnivores were more prevalent in the least disturbed perennial systems. Minoshima *et al.* (2007) reported, that during a one year field trial in California, the soil food web, as indicated by the nematodes, did not become more complex with no-tillage and continuous cropping, contrary to expectations and possibly because higher trophic level nematodes have been eliminated after decades of cultivation.

- The population numbers of bacterivores and fungivores were quite high in some of the treatments, making a discussion of the treatment effects on their incidence very relevant. The population numbers of these nematodes also give an indication of the dominant decomposition pathway in the soil (Pradhan *et al.*, 1988), which in turn will give an indication of the diversity of soil organisms in a particular soil and, hopefully, an indication of increased suppression of herbivorous nematodes in the different treatments (Pattison *et al.*, 2006).

Table 8. Treatment effects on the incidence of nematode species 64 DAP.

Nematodes		Incidence ^x																			
		ANM ^z	ANP	ANM+OB	BF	BO	C	CAM	CR	CRI	ECO	FIM	FP	GLIO	MBM	MBP	OB	SR	TM	TP	LSD
Bacterivores (Total)		356.7ab	278.3ab	285.0ab	356.7ab	868.3a	163.3b	868.3a	355.0ab	173.3b	158.3b	493.3ab	390.0ab	445.0ab	276.7ab	365.0ab	375.0ab	538.3ab	283.3a	323.3ab	619.0
Fungivores (Total)		218.3b	266.7b	165.0b	208.3b	450.0b	168.3b	270.0b	185.0b	146.7b	148.3b	160.0b	226.7b	255.0b	128.3b	246.7b	378.3b	205.0b	1036.7a	383.3b	391.4
Omnivores (Total)		8.3a	0.0a	6.7a	0.0a	5.0a	0.0a	0.0a	0.0a	0.0a	1.7a	0.0a	6.7a	0.0a	5.0a	5.0a	0.0a	5.0a	0.0a	5.0a	NS
Predators (Total)		1.7a	5.0a	1.7a	8.3a	8.3a	5.0a	26.7a	3.3a	15.0a	20.0a	8.3a	28.3a	1.7a	6.7a	13.3a	28.3a	10.0a	20.0a	18.3a	NS
Beneficial (Total)		551.7cd	523.3d	423.3d	345.0d	1161.7ab	246.7d	1131.7ac	408.3d	278.3d	285.0d	631.7b-d	483.3d	603.3b-d	381.7d	543.3d	725.0b-d	728.7b-d	1693.3a	613.3b-d	587.6
Herbivores (Total)		1741.7fj	1945.0d-j	1188.3g-j	3691.7a-d	2930.0b-g	1891.7e-j	4370.0ab	2886.7b-h	781.7ij	1121.7h-j	3301.7a-f	3656.7a-e	2920.0b-g	276.7i	223.3j	2065.0d-h	3666.7a-c	1486.7g-j	1228.3g-h	1787.3
<i>Cricemoides sphaerocephalus</i>		1.7b	0.0b	3.3b	56.7a	1.7b	3.3c	8.3b	0.0b	1.7b	8.3b	8.3b	21.7ab	0.0b	0.0b	1.7b	0.0b	0.0b	1.7b	5.0b	37.47
<i>Dorylaimelus</i> sp.		1.7bc	0.0c	0.0c	16.7ab	3.3bc	25.0a	0.0c	0.0c	0.0c	0.0c	0.0c	6.7bc	0.0c	0.0c	0.0c	0.0c	0.0c	0.0c	0.0c	16.2
<i>Helicotylenchus ditylensis</i>		630.0d-h	845.0d-h	520.0e-h	748.3d-h	1636.7b-d	950.0d-h	2518.3ab	1156.7d-f	236.7f-h	443.3e-h	1301.7c-e	1555.0b-d	1026.7d-h	56.7gh	35.0h	1065.0d-g	2200.0ab	425.0e-h	445.0e-h	1022.2
<i>Scutellonema brachyurum</i> , <i>S. unum</i>		16.7c	5.0c	23.3c	193.3a	13.3c	226.7c	26.7c	26.7c	23.3c	31.7c	131.7ab	160.0a	16.7c	1.7c	1.7c	36.7bc	3.3c	45.0bc	5.0c	96.6
<i>Longidorus pisi</i>		0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	40.0a	0.0a	0.0a	5.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	40.0a	0.0a	0.0a	NS
<i>Meloidogyne</i> spp.		26.7c	120.0a	56.7c	373.3ab	171.0c	115.0e-c	138.3a-c	148.3a-c	81.7c	121.7	140.0a-c	391.7a	135.0e-c	288.3a-c	110.0bc	278.3a-c	95.0c	101.7bc	90.0c	276.9
<i>Paratrichodorus minor</i>		903.1b-d	795.0b-d	521.7b-d	2068.3a	1020.0a-d	648.3b-d	1446.7a-c	1370.5a-c	398.3cd	460.0cd	1536.7a-c	1651.7ab	1651.7ab	51.7d	63.3d	555.0b-d	890.0b-d	850.0b-d	613.3b-d	1147.0
<i>Pratylenchus brachyurum</i> , <i>P. zeae</i>		161.7b	180.0b	63.3b	245.0b	83.3b	23.3b	191.7b	180.0b	40.0b	51.7b	183.3b	303.3ab	88.3b	13.3b	11.7b	130.0b	638.3a	63.3b	65.0b	372.6
<i>Rotylenchulus pervus</i>		0.0b	0.0b	0.0b	0.0b	0.0b	0.0b	8.3a	0.0b	0.0b	0.0b	0.0b	1.7b	0.0b	0.0b	0.0b	0.0b	0.0b	0.0b	0.0b	6.1
<i>Tylenchorhynchus brevilineatus</i>		13.0bc	10.2bc	14.7bc	7.0c	22.1bc	6.5c	15.7bc	10.2bc	16.4bc	12.2bc	11.1bc	8.3c	14.2bc	41.5a	42.4a	13.4bc	11.1bc	26.3ab	16.4bc	17.0
Bacterivores (%)		8.5e-e	12.1e-e	12.5e-e	4.9e	9.3e-e	6.4de	4.9e	5.4e	12.7c-e	10.7c-e	4.0e	5.9e	7.6e-e	20.2a-c	28.9ab	13.0e-e	4.6e	30.2a	19.9e-d	13.7
Fungivores (%)		0.3ab	0.0b	0.4ab	0.0b	0.07ab	0.0b	0.0b	0.0b	0.0b	0.1ab	0.1ab	0.0b	0.0b	0.7ab	0.9a	0.0b	0.0b	0.0b	0.2ab	0.9
Omnivores (%)		0.07c	0.3a-c	0.1c0	0.1a-c	0.3e-c	0.2a-c	0.4e-c	0.1bc	1.5a	1.1a-c	0.2a-c	0.6a-c	0.1c	0.7a-c	1.5ab	1.3a-c	0.2a-c	0.6a-c	1.1a-c	1.4
Predators (%)		27.7d-g	28.5d-g	29.7d-g	13.4g	37.0c-e	13.1g	26.7d-g	21.4e-g	41.9cd	27.2d-g	22.7e-g	17.6fg	32.0c-f	67.3ab	75.4a	30.0d-g	18.8fg	67.0ab	49.0bc	18.0
Beneficial (%)		78.1e-c	77.4e-c	72.3a-c	88.0a	68.1bc	86.9a	79.0a-c	84.3	69.4bc	75.8a-c	84.7ab	85.1ab	78.1a-c	36.9d	26.2d	72.4a-c	84.0ab	42.9d	62.4c	17.04

^x Incidence = number of nematodes extracted from 20 g roots and 250 cm³ soil.

^y Means in a row followed by the same letter do not differ significantly (P = 0.05).

^z See Table 2 for treatment abbreviations.

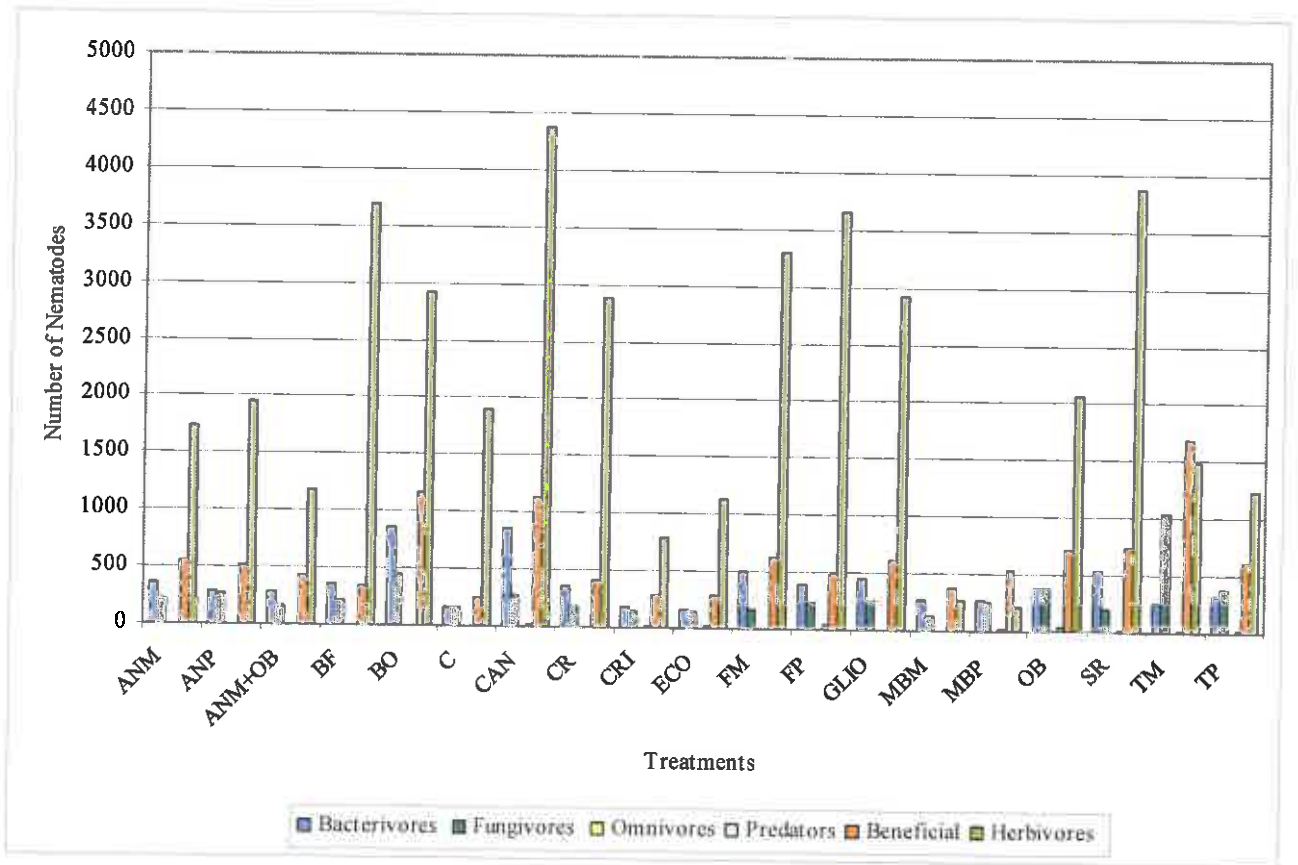


Fig 1. Number of nematodes found in soil and roots 64 DPA.

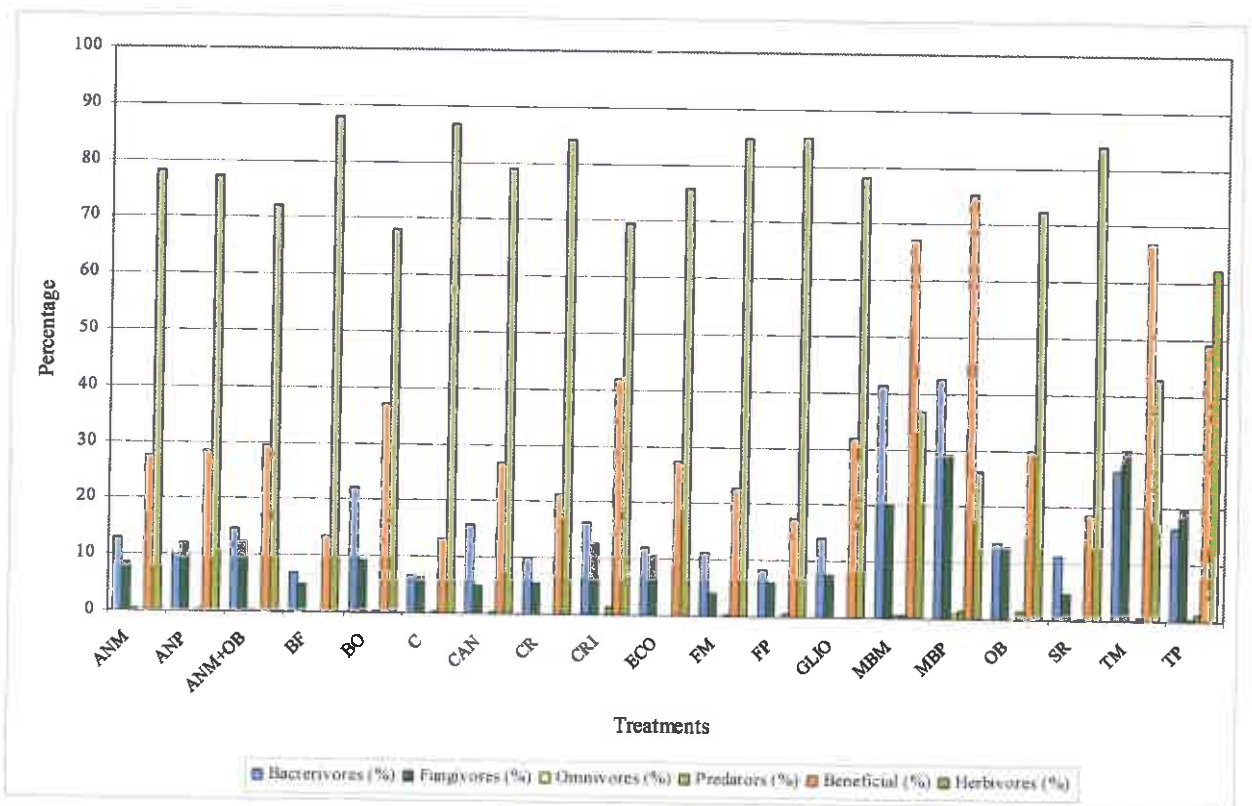


Fig. 2. Incidence of nematodes in soil and roots DPA.

Microbial diversity and activity in soil

An overview on microbial diversity and activity in soil as published by previous researchers in other countries has been given in detail in previous reports and will not be repeated here.

ROTATIONAL EFFECTS

Growth, plant and soil analysis

From the yield data presented in Table 9 it is apparent that rotational effects noted this season were similar to those recorded in the 2008/2009 season. At the first sampling 28 DAP, the BF treatment was significantly superior to the control (C) and the maize fallow (FM) was nearly so. This is considered to have probably resulted from more favourable soil temperatures in reduced-cover plots during the early part of the season (Table 10) and was visually clearly evident from the date of emergence. It is noteworthy that by 64 DAP this advantage had disappeared (Table 11) and that by 140 DAP, the BF and FM treatments were significantly worse than the control (Table 12). This has happened previously and from Tables 11 and 12 is clearly reflected in terms of soil moisture content. The rapid decline in the performance of these two treatments from 64 DAP was dramatic and in terms of final grain yield the control (C) out-performed both by approximately 1500 kg/ha.

Table 9. Treatment effects on plant mass at three sampling stages (g/6 plants 28 DAP and g/8 plants 64 and 140 DAP) and grain yield (kg/ha).

Treatment*	28 DAP	64 DAP	140 DAP	GRAIN YIELD
ANM	28.87	9020	9070	15450
ANP	30.38	9280	9050	15650
ANM+OB	30.75	9200	9930	15203
BF	38.07	7430	7080	12453
BO	23.11	8200	9830	14105
C	26.41	7950	10030	14066
CAN	34.27	8970	9680	14324
CR	33.22	9080	9480	14798
CRI	38.32	8600	8400	13230
ECO	27.89	8630	9580	14044
FM	34.13	7720	8430	12637
FP	33.74	8170	8500	14112
GLIO	27.52	8550	9100	13744
MBM	47.76	8380	6550	13129
MBP	41.56	8870	9450	14640
OB	27.54	8400	9480	14111
SR	24.12	9130	10220	14365
TM	45.95	8020	6320	12783
TP	36.25	8680	9180	14215
LSD (0.05)	8.12	1070	1050	802

* See Table 2 for description of treatments.

Since the biomass load in canola (CAN) and crambe (CR) plots was lower than that in the control (C) plots, a similar cover effect may possibly have partially resulted in the rotational benefits of these rotations 28 DAP (Table 9 & 10). However, the yield benefit due to canola and crambe persisted 64 DAP and yields obtained in these treatments 140 DAP and at harvest, were not significantly different from those in control plots. This issue will be discussed further in the section to follow, but crown and root rot severity data presented in Table 11 suggest that in this case we are seeing a true rotational benefit as a result of disease suppression.

The effects of stooling rye (SR) and black oat (BO) were markedly different from the effects already discussed. At 28 DAP these two treatments resulted in yields which were no better than the control (C). Previously noted allelopathic effects cannot be discounted, but, since the BO and SR plots carried biomass loads equal to the control, it is likely that cover effects in these treatments would have very closely matched those in control plots (Table 10). Interestingly, 64 DAP the yields in SR plots were significantly superior to the control (C), but those of BO plots were not (Table 9). Data in Tables 11 and 12, also to be discussed in more detail below, strongly suggest that these treatments influenced disease severity, but it is known that the allelopathic effects of BO exceed those of SR. At 140 DAP and at harvest there were no statistical differences between these treatments, however.

Of particular relevance to this section on rotational effects is the fact that in the exceptionally good 2007/2008 season, all alternative rotations to wheat, with the exception of BF, which had not yet been introduced, significantly out-performed the control through to grain yield (Lamprecht *et al.*, 2008). This is clearly evident in Table 13 and illustrates the complexities introduced by the benefits of cover in seasons of moisture stress. It appears that in three out of four seasons thus far, the benefits of enhanced moisture-use efficiency as a consequence of cover – reduced evaporative moisture loss from the soil surface, increased earthworm numbers and, thus, soil macroporosity and infiltration-rate – overwhelmed the negative effects of soilborne diseases. If this is so, it is likely that rotational benefits would be more clearly expressed under full irrigation.

Table 10. Treatment effects on relative yield 28 DAP and soil temperature and moisture content 42 DAP.

Treatment ^a	% Yield	Soil Temp °C	Soil Moist. %
MBM	100	29.6	5.6
TM	96	29.0	4.8
MBP	87	27.9	11.8
CRI	80	-	-
BF	80	29.3	12.9
TP	76	28.5	9.6
CAN	72	27.8	17.8
FM	72	28.6	18.6
FP	71	28.1	23.4
CR	70	28.6	16.6
ANP	64	28.5	15.2
ANM+OB	64	-	-
ANM	60	29.9	7.5
ECO	58	-	-
GLIO	58	-	-
OB	58	-	-
C	52	26.8	20.6
SR	51	28.4	24.0
BO	48	27.6	22.3
LSD (0.05)	17	NS	3.8

^a See Table 2 for description of treatments.

Table 11. Treatment effects on relative yield, crown and root rot severity ratings 64 DAP, on earthworm counts 60 DAP, and soil temperature and moisture content 73 DAP.

Treatment ^a	% Yield	Crown Rot Severity	Root Rot Severity	Worm Count Per m ²	Soil Temp °C	Soil Moist. %
ANP	100	0.71	1.75	75	25.9	12.6
ANM+OB	99	0.42	1.42	63	-	-
SR	98	0.71	1.75	141	26.5	15.5
CR	98	0.67	1.54	92	25.8	16.3
ANM	97	0.50	1.42	41	27.4	6.0
CAN	97	0.63	1.63	106	26.5	14.4
MBP	96	0.63	1.63	35	25.8	12.7
TP	94	0.75	1.71	83	25.6	12.8
ECO	93	0.88	2.00	80	-	-
CRI	93	0.58	1.42	91	-	-
GLIO	92	0.79	1.92	110	-	-
OB	91	0.88	2.21	109	-	-
MBM	90	0.50	1.33	9	26.4	5.8
BO	88	0.63	1.75	150	25.4	16.2
FP	88	0.83	2.00	67	25.7	16.6
TM	86	0.79	1.79	17	26.2	5.5
C	86	1.25	2.33	103	26.1	15.4
FM	83	0.88	1.83	21	25.9	9.9
BF	80	1.17	2.33	7	26.4	11.5
LSD (0.05)	12	0.23	0.41	44	2.5	3.6

^a See Table 2 for description of treatments.