Final Report

REPORT ON PROJECT ENTITLED "THE ROLE AND IMPORTANCE OF SOILBORNE DISEASES AND MICROBIAL DIVERSITY ON MAIZE PRODUCTION AS WELL AS THE INTERACTIVE EFFECTS OF CROP ROTATION AND BIOCIDES"

Submitted on behalf of
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FRONT PAGE

Control on left and
methyl bromide treated on right
(Lamprecht *et al.*, 2006)

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SC Lamprecht[1], MPW Farina[2], GR Thibaud[3], M Marais[4], JH Habig[4], JF Bloem[4] and A. Swart[4]

[1]ARC-Plant Protection Research Institute, Private Bag X5017, Stellenbosch, 7599.

[2]Omnia Fertilizers, 27 Drew Avenue, Howick, 3290.

[3]KZN Department of Agriculture and Environmental Affairs, Cedara College, Private Bag X 9059, Pietermaritzburg, 3200.

[4]ARC-Plant Protection Research Institute, Private Bag X134, Queenswood, 0121.

E X E C U T I V E S U M M A R Y

A field trial was conducted in KwaZulu-Natal (KZN) to determine the effects of alternative winter crops and soil treatments on no-till maize yields, soilborne diseases of maize (fungi and nematodes) and microbial diversity and activity. A seed treatment trial was also conducted in a growth room to establish the effect of the fungicides Apron (metalaxyl), Thiulin (thiram), Rizolex (tolclofos methyl), (singly and all three combined in a single and double dosage), Celest (fludioxonil/metalaxyl)) and Dynasty (fludioxonil/mefenoxam/azoxystrobin) on seedling survival and seedling growth. The field trial was conducted at Winterton, and consisted of fifteen treatments, which included the winter crops canola (CAN), crambe (CR), black oats (BO), wheat without tillage(C), wheat with tillage (T), soyabean-wheat (SW), fallow (Maize fallow =MF and soyabean fallow =SF); soil treatments with anhydrous ammonia (AN) and methyl bromide fumigation (MB), a nematicide (N), and the biocontrol agents Fungimax + Organoboost (OB), Spin + Webstarter (S), Eco-T (ECO) and Extrasol (EXT). The maize cultivar PHI 32D96B was used for both the field and growth room study. In the field study, weeds and foliar diseases were chemically controlled. Ten whole plant samples were collected from each plot 21, 70 and 100 days after planting. The parameters evaluated included soil and plant chemical composition, and the

incidence of nematodes at 21 and 100 days, plant mass, crown and root rot severity, incidences of specific fungi, and microbial diversity and activity at each of the three growth stages and grain yield at harvest. Sucker counts were conducted 46 days after planting. Selected treatments were also sampled prior to planting to determine the incidence of nematodes and to assess microbial diversity and activity prior to the application of biocontrol agents, nematicide, and anhydrous ammonia. Chemical analyses of soil and plant material showed that, while treatments had not affected soil properties, there had been marked effects on plant composition following the use of AN. Depressions in the uptake of K, Ca and Mg were very marked at 21 days and the effects on Ca and Mg persisted for at least 100 days. This is consistent with the build-up of NH_4-N, which accompanies NH_3-gas application. The longevity of this effect was clearly evident in terms of root and crown rot suppression and final grain yield, something which is extremely encouraging from the point of view of effective and economical soilborne disease control. There were also very marked increases in plant Mn content, another factor know to suppress diseases in many plants. The final grain yield of AN plots was more than 1000 kg/ha higher than that of any other treatment and over 2300 kg/ha higher than those treatments without trash cover, which included methyl bromide fumigation. Somewhat surprisingly, no-till plots following wheat also significantly out-yielded treatments without cover (MB, T, MF & SF). This possibly resulted from moisture and temperature effects on disease development, and has identified an issue of critical importance to the development of no-till farming, which demands further investigation and validation. As far as is known, these findings are unique and could play a critical role in switching farmers with confidence from conventional to no-till systems. In terms of final grain yield, none of the other alternative strategies of soilborne disease control proved superior to the control (maize after wheat). The effects of Eco-T, however, certainly warrant further investigation. This product resulted in very beneficial results up until the 100 day after planting stage and at harvest it was still the third best performing treatment. Sucker counts conducted 46 days after planting related remarkably well to yield and root rot 70 and 100 days after planting. Clearly, suckering is an index of root health and plant vigour, which should not be ignored in other research of this nature. The lowest crown and root rot severities were recorded for AN, CAN, MB and the T treatments and the highest for the BO, C, N and OB treatments. Crown and root rot severities were significantly negatively correlated with the growth of plants. Fungi most frequently

isolated from diseased crown and root tissue were *Fusarium equiseti*, *F. graminearum*, *F. nygamai*, *F. oxysporum*, *F. proliferatum*, *F. solani*, *F. subglutinans*, *Pyrenocheata terrestris*, and *Trichoderma* spp. Of these, *Trichoderma* spp. followed by *F. oxysporum*, *F. graminearum* and *P. terrestris* were predominant. Of the fungi isolated, *F. graminearum*, and *P. terrestris* 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 and treatments. Incidence of *F. graminearum* increased from the beginning to the end of the season, whereas *P. terrestris* was more prominent at the second compared to the first and third sampling times. The lowest incidences of *F. graminearum* in roots were recorded for the AN, CAN, CR, MB, MF, SF and T treatments. There were significant positive correlations between the incidences of *F. graminearum* and crown and root rot severity. Treatments had no effect on the incidences of *P. terrestris* in crowns and roots. Plant-parasitic nematodes isolated from the soil and roots were *Criconemoides sphaerocephalus*, *Helicotylenchus digonicus*, *H. dihystera*, *H. paraplatyurus*, *H. pseudorobustus*, *Longidorus pisi*, *Meloidogyne incognita*, *M. javanica*, *Paratrichodorus minor*, *Pratylenchus brachyurus*, *P. zeae*, *Rotylenchulus parvus* and *Scutellonema brachyurus*. Of these *H. dihystera*, *S. brachyurus* and *R. parvus* were predominant. Previous studies in South Africa proved that maize is a good host for all the plant-parasitic nematodes found during the study. The incidences of the nematodes were affected by sampling time and some of the treatments. The incidence of most of the plant-parasitic nematodes increased over the season with the *Helicotylenchus* spp., *S. brachyurus* and *R. parvus* reaching the highest numbers in the soil and *P. brachyurus* and *P. zeae* the highest population numbers in roots. The lowest incidences of plant-parasitic nematodes were recorded in the MB treatments and the highest in the SF treatments. Microbiological methods used to assess soil microbial diversity and activities were able to distinguish between different crop cover treatment groups. During the season, the biodiversity indices of full cover treatments were slightly higher than that of the partial and no cover treatments. These techniques proved once again to be cost-effective and useful as soil quality indicators. The seed treatment trial showed that fungicidal seed treatment did not significantly affect survival, but the combination of Apron, Thiulin and Rizolex (targeting fungi such as *Fusarium* spp., *Pythium* spp. and *Rhizoctonia* spp.) significantly increased plant height compared to the control (untreated seed),

indicating that a complex of fungi are involved in root rot of maize seedlings. Although the MB treatment did not perform as well as during the previous season, due probably to moisture stress and high temperatures, this study showed that there are alternative crops that performed the same as wheat with regard to grain yield, but with decreased incidences of important pathogens such as *F. graminearum*. It also confirmed that crown and root rot negatively impact on growth of maize plants and that crown and root rot are caused by a complex of organisms, which include both fungi and nematodes. Furthermore, it appears that *Trichoderma* spp. play an important role in control of soilborne pathogens of maize, but this needs to be investigated in more detail. The results obtained so far also showed that a number of control measures need to be combined in order to develop a strategy to manage soilborne diseases of maize in a sustainable manner. It is, however, crucial that the role of each pathogen in the disease complex and the different interactions between them should be determined under controlled environmental conditions. It is also essential that the field study conducted this season should be repeated for at least one more season to account for different climatic conditions.

I N T R O D U C T I O N

The importance of soilborne diseases in maize, caused by a variety of pathogenic fungi and parasitic nematodes, is widely recognised internationally and there is an abundance of literature on the topic (Warren & Kommedahl, 1973; Young & Kucharek, 1977; Sumner & Bell, 1982; Scholte, 1987; Ramsey, 1990; Howard *et al*., 1998; Zhang *et al*., 1998; White, 1999).

Most published research, however, has addressed the effects of soilborne diseases in conventional tillage systems and there is strong circumstantial evidence to indicate that such diseases have an even greater detrimental effect under no-till systems (Deep & Lipps, 1996; Mannering & Griffiths, 2000; Sumner *et al., 2002),* systems currently being promoted in South Africa and elsewhere in the interests of soil conservation, enhancing soil biodiversity, reducing production costs, and conserving non-renewable energy resources (Unger, 1992). It is currently estimated that there are 25 million hectares under no-till in the USA, over 42 million hectares in South America and some 300 000 hectares in South Africa (Paterson, 2007).

In this country, the situation is aggravated by the fact that, although a number of fungi and nematodes have been associated with diseased maize roots, information on the relative importance of these pathogens is limited (Du Toit, 1968; Kruger, 1970; Walters, 1979; Scott, 1982; Deacon & Scott, 1983; Chambers, 1987a,b; Smit, 1998; Smit *et al.,* 1997). Field experiments designed to quantify the yield losses likely to result from soilborne diseases are virtually non-existent, and work that was conducted toward this end in KwaZulu-Natal failed to identify the pathogens involved (Channon & Farina, 1991). Moreover, research into the effects of soilborne diseases in no-till systems has only recently been initiated (Lamprecht *et al.,* 2006). Further complicating this deficiency is the fact that the dominant species of pathogenic fungi and parasitic nematodes varies considerably with locality and overseas findings cannot simply be adapted to local conditions. Many of the primary nematodes and fungi in the USA, for example, have not been shown to be problematic in this country. Indeed, it is highly probable that, even within South Africa, the dominant suite of fungi and nematodes attacking maize will differ appreciably across soil types and bioclimatic zones (Lamprecht, 2007).

There is also a need to quantify the effects of cropping systems on disease incidence. The negative effects of monoculture are widely recognised and the importance of proper crop rotations to reduce yield losses due to soilborne diseases in conventional cropping systems was reported on several decades ago in the USA (Williams & Schmitthenner, 1963). Work carried out in this country has demonstrated the appreciable ameliorative effects of maize-soyabean rotations, but here, too, under conventional tillage (Farina, 2000) and, moreover, without any direct measure of the effects of soyabean on the populations of disease organisms. Significantly, however, 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 very limited range of crops that can be profitably produced during winter months and wheat, the most popular winter crop rotated with maize is susceptible to pathogens, such as *Fusarium graminearum*, that also attack maize roots, stalks and cobs (White, 1999). Wheat-maize rotations consequently result in a build-up of such pathogens and detrimental effects on both crops (Schaafsma *et al*,. 2005). This has led to the frequent introduction of trash burning after harvesting maize in an effort to reduce the problem of "scab" in following wheat, 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 to maize offers a partial solution, but can result in increased incidence of "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. Only then will it be possible to asses the economic viability of potential intervention strategies.

Other methods of possibly controlling soilborne diseases include the use of costly chemicals, employing biocontrol agents, of which there is currently a plethora of largely untested products being marketed to farmers, the introduction of fallows, seed treatments with fungicides, and the use of anhydrous ammonia as an alternative

nitrogen source. This product has long been known to suppress certain fungal and nematode populations in a range of crops and soils (Eno *et al*., 1955), is currently quite widely used in South Africa because of cost benefits it offers and, if shown to be effective in maize at economic application rates, could provide an invaluable intervention strategy.

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 & 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 populations, 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 content (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 increase 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 the rhizosphere and improved physical condition of soils in crop rotations were observed particularly when rotations contained legume species (Miller & Dick, 1995a, b).

Findings obtained in a preliminary study conducted in the Winterton / Bergville area during the 2005 / 2006 season clearly demonstrated that soilborne diseases were depressing maize yields in irrigated systems where maize followed winter wheat (Lamprecht *et al*., 2006). At Winterton, the response to methyl bromide fumigation, a treatment used as an experimental tool in order to establish yields potentially attainable in the absence of soilborne diseases, was particularly marked. Yields in methyl bromide treated plots were, on average, two t/ha (14 %) higher than the other treatments. Two biocontrol agents (Eco-T and Extrasol) proved to have no effect on grain yield or the incidence of root diseases. A large number of pathogens was isolated, but those which appeared to be of primary importance were *F. graminearum*, a pathogen common to both maize and wheat, *Pyrenochaeta terrestris* and *Pythium* spp.

Having established that soilborne diseases were meaningfully depressing yields, the challenge was to identify economic and practical ways of simulating the effects of soil fumigation. One possibility was to use alternative rotational crops to wheat and another was to test the effects of soyabean prior to wheat. It also seemed desirable to measure the effects of a winter fallow after maize and soyabean, a wider range of products marketed as biocontrol agents, and anhydrous ammonia as a nitrogen source. Toward this end, an experiment was designed, which would hopefully provide the information required. This experiment, generously funded by the Maize Trust, the

KwaZulu-Natal Department of Agriculture and Environmental Affairs, and Omnia Fertilizers, was initiated in the winter of 2006.

Previously, only treatment effects on fungal root disease incidence and microbial diversity were related to yield. Exploratory data, however, indicated that parasitic nematode populations were high and, since nematode damage has been shown to provide root access to certain fungal pathogens, it seemed necessary that nematode counts be conducted in order to accommodate a potentially serious confounding factor. In addition to the field trial, a seed treatment trial was considered necessary in order to determine whether seed treatments different to those currently registered might not also help to eliminate or reduce the negative effects of soilborne diseases.

The primary objectives of this project were to (1) identify and quantify the dominant soilborne fungal pathogens and parasitic nematodes that occur in maize following winter wheat in no-till irrigated systems, (2) assess the effects of fallow breaks after maize and soyabean and of alternative winter crops to wheat, (3) test the efficacy of four biocontrol agents and anhydrous ammonia, (4) compare tillage after wheat in the place of no-till, (5) measure the effects on maize growth and grain yield, and on microbial diversity in the soil and (6) examine the effects of alternative seed treatments. Methyl bromide fumigation was employed in order to provide the targeted base line to be simulated.

MATERIALS AND METHODS

The treatments created during the previous winter and summer seasons, or imposed shortly before or after planting during the 2006/2007 season are shown below in Table 1. Points following immediately below the table provide information regarding the rationale behind their selection.

Table 1. Treatments included in the field trial.

Treatment Code	Treatment	Previous Summer Crop	Winter Crop	Current Crop
AN	Anhydrous ammonia	Maize	Wheat	Maize
BO	Black oats	Maize	Black oats	Maize
C	Control	Maize	Wheat	Maize
CAN	Canola	Maize	Canola	Maize
CR	Crambe	Maize	Crambe	Maize
ECO	Eco-T	Maize	Wheat	Maize
EXT	Extrasol	Maize	Wheat	Maize
MB	Methyl bromide	Maize	Wheat	Maize
MF	Maize fallow	Maize		Maize
N	Nematicide	Maize	Wheat	Maize
OB	Fungimax + Organoboost	Maize	Wheat	Maize
S	Spin + Webstarter	Maize	Wheat	Maize
SF	Soyabean fallow	Soyabean		Maize
SW	Wheat after soyabean	Soyabean	Wheat	Maize
T	Tilled	Maize	Wheat	Maize

The rationale for inclusion of these treatments is as follows:

AN	–	Anhydrous ammonia is a popular N source and there is evidence that it has biocidal properties. Not only is NH_3 gas highly toxic, but plant uptake of NH_4-N ($NH_3 + H_2O \rightarrow NH_4OH$) has been shown to depress the incidence of many fungal diseases (Huber, 1991) and the intermediary product of nitrification (HNO_2) is toxic (Tenuta & Lazarovits, 2002).
BO	–	Black oats is considered in Brazil to be an excellent rotational crop and is reputed to have allelopathic properties.
C	–	Control plot against which to measure treatment responses.
CAN	–	Canola is a possible alternative winter crop to wheat and has economic potential.
CR	–	Crambe is another alternative winter crop to wheat, which has economic potential.
ECO	–	Eco-T is a currently marketed biocontrol agent.
EXT	–	Extrasol is a currently marketed biocontrol agent.
MB	–	Methyl bromide eliminates soilborne diseases and is used to provide a base line of the potential maize yield attainable.
MF	–	Maize fallow to determine whether omitting wheat provides any benefit, as fallows are generally considered to assist in disease control.
N	–	Nematicide to determine whether there are interactions between nematodes and fungal root disease incidence.
OB	–	Fungimax + Organoboost, a combination biocontrol agent, is currently marketed to enhance fungal and bacterial populations.
S	–	Spin + Webstarter, another biocontrol agent, is currently marketed to achieve the same effects as OB.
SF	–	Soyabean fallow to determine whether there is a benefit from soybean if wheat is omitted prior to planting the next summer crop.
SW	–	Soyabean-wheat-maize rotation to determine whether the benefits of soyabean can work through a winter wheat crop and benefit following maize.
T	–	Tilled without MB to measure the contribution of tillage effects *per se*.

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. The site has been under no-till for many years, but is outside the pivot circle and had not previously been cropped during the winter. In order to grow the winter crops needed to test the rotational effect (wheat, canola, crambe and black oats) in double-cropped systems, irrigation was provided with a movable system kindly provided and operated by Mr Muirhead. Chemical properties of the experimental soil are provided in Table 2.

Table 2. Selected physical and chemical properties of the experimental soil (0-150 mm).

	Exchangeable Cations								
Soil Form	K	Ca	Mg	Al+H	Zn	Mn	pH(KCl)	Clay	Organic Carbon
	cmol/L				mg/L			%	
Hutton	0.39	7.10	1.86	0.08	8	12	4.81	45	2

A portion of the site shortly before planting the maize crop is shown in Fig. 1. Clear plots are those in which wheat had been treated previously with glyphosate to facilitate rotovation and MB fumigation (white pegs), and the creation of tilled, but unfumigated plots (no pegs). All plant material was incorporated. Methyl bromide was applied at 1000 kg/ha. Green plots are those that had been planted to canola (*Brassica napus)* or crambe (*Crambe abyssinica*) instead of wheat, and the plot adjacent to that fumigated and between the green plots in the foreground is one that had been planted to black oats (*Avena strigosa*), an extremely popular rotation crop in Brazil. Not shown are plots that were left fallow after maize or soyabean grown the previous summer (2005/2006). All the other plots were under wheat that had just been treated with glyphosate.

Fig. 1. A view of the experiment shortly before planting maize.

The experimental site was specifically not combined in order to minimise across-plot contamination of fungi such as *Fusarium graminearum,* which affects both wheat and maize, and was directly planted to the maize cultivar PHI 32D96B at a population of 65 000 plants per ha on the 20th November 2006, approximately 14 days after the photograph shown in Fig. 1 was taken. An image of the trial immediately after planting is shown in Fig. 2.

Fig. 2. A portion of the experimental site immediately after planting.

At planting, 300 kg/ha of single superphosphate was applied in the band. No potassium was required. All plots, other than the three which received nitrogen in the form of anhydrous ammonia (AN), were fertilized with 200 kg/ha of nitrogen in the form of LAN. Plots which received AN at the same rate of nitrogen were cut and raked before the gas was applied, because of the risk of stover disrupting the operation. This application is depicted in Fig. 3. Gas is escaping as the tynes penetrate the soil. LAN was hand applied to plots on the 16th of November and AN a day later. Plot dimensions were 7.28 m 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 mown 1-m pathway and there was an additional border area of 1 m at both ends of each plot.

Fig. 3. Applying anhydrous ammonia.

Immediately after planting, the biocontrol treatments (ECO, EXT, OB & S) were sprayed over the row in a 10-cm wide band at the recommended rate in the equivalent of 1 450 L/ha of water. The nematicide, Crop Guard (N), was similarly applied in a 50-cm band over the row. One month after planting, all the biocontrol products were reapplied in the same fashion and at the same rates as those previously used. This was done, because some purveyors of the products recommended that this should be done and it would have been unfair to prejudice the others.

Soil samples were collected for microbial diversity analysis just prior to planting. Thereafter, rhizosphere soil was collected from each plot at the three stages during which root samples (10 plants per plot) were collected for root disease ratings and fungal isolations. At each sampling (11/12/06, 29 & 30/1/07 and 26 & 27/2/07), sub-samples of soil were collected for microbial diversity analysis and at the first and third sampling sub-samples were also collected for chemical analysis and nematode counts and identification. At each sampling, the mass of above-ground plant parts was determined and at the first and second sampling (silking stage) either whole plants or leaf samples (leaf opposite and below the primary ear) were collected for chemical analysis. Standard procedures used at Cedara (Farina & Channon, 1988) were employed in chemically analysing soil and plant material. Sucker counts were done 46 days after planting in order to assess whether the occurrence of tillers was related to treatment.

Throughout the period from planting to acquisition of the third set of samples, stringent precautions were taken to minimise across-plot contamination. Shoes were disinfected in Sporekill prior to entering each plot and forks used to remove plants were thoroughly scrubbed and washed in a 70 % ethanol solution. Only the 1-m pathways were used when moving irrigation equipment.

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.

Disease ratings were done on arrival at the ARC soilborne disease laboratories in Stellenbosch. 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. All fungi were identified and recorded.

The soil and root samples for nematode analyses were transported in cooler boxes to the laboratory and stored at 12 ºC. Each soil sample was thoroughly mixed before a 250 g sub-sample was taken from it. Nematodes were extracted according to the sieving-centrifugation-flotation method (Kleynhans, 1997). The root samples were rinsed free from soil and cut into smaller pieces (± 20 mm), the material (20 g) was shredded in a food blender and washed trough 150 µm, 45 µm and 38 µm aperture sieves. The residue on the sieves was transferred to 100 ml centrifuge tubes and the nematodes extracted according to the centrifugation-flotation procedure. The nematode suspension was counted 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. Bacteriophagous, fungiphagous and predatious nematodes were counted together as non plant-parasitic nematodes.

For quick identification to species level, the nematodes were mounted on temporary slides and identified with a research microscope. For further studies, specimens were preserved in FPG (distilled water, 4 % formaldehyde, 1 % propionic acid, glycerine and picric acid) and mounted in anhydrous glycerine on Cobb aluminium double cover slip slides (Netcher & Steinhorst, 1969; Kleynhans, 1977). These slides were deposited in the National Collection of nematodes.

Soil samples for analyses of functional diversity of microbial populations were stored at ±5 °C prior to analysis, and samples for enzyme activities were dried at 40 °C/48 h, sieved (< 2 mm) and also stored at ±5 °C. Soil samples were diluted (1:3 000) and plated onto Biolog EcoPlates™ (Biolog® Inc., Hayward, USA) containing 31 carbon sources and a control well, in triplicate. The plates were incubated at 25 °C and optical density was measured twice daily over a period of 5 days at 590 nm. 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 ß-Glucosidase, alkaline phosphatase , acid phosphatase, and urease activities in the soil samples were assayed. ß-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.

Growth Room Trial

Maize seeds (cv. PHI 32D96B) were treated with Apron at 0.5 ml/kg (0.326 g a.i./kg), Thiulin at 2 g/kg (500g a.i./kg), Risolex at 1.5 g/kg (500 g a.i./kg), a combination of the above three fungicides at the dosage specified (Comx 1) and at double the dosage (Comx 2), Celest at 1 ml/kg (2.5 g a.i./L) and Dynasty at 2.5 ml/kg (125 g a.i./L). Untreated seeds served as the control.

Soil (approximately 10 kg) was collected from each of the field plots subjected to treatments BO, C (20 kg), CAN, CR, MB, MF, SF, SW and T and transported to Stellenbosch. Crop Guard was applied to the C soil (10 of the 20 kg) at 0.1328 ml/700 g soil to simulate the N treatment in the field. There were three replicates of each of the soil treatments. Eight pots 13 cm in diameter were each filled with 700 g of soil from each replicate of each treatment and planted to the seed treated with the eight different seed treatments at one pot/seed treatment. Ten seeds were planted per pot. Pots were kept in a growth room at 26 °C day and 18 °C night temperature with a 12-h photoperiod. Seedling survival and plant height and dry mass were determined 2 weeks after planting.

Statistical Analyses

The experimental layout used in the field study was a complete randomised block design consisting of 15 treatments in three replications. The experimental layout used in the growth room study was a randomised block design consisting of ten soil treatments and eight seed treatments in three replications. All acquired data were statistically analysed by the ARC statistician in Stellenbosch.

Non-parametric statistical analyses were performed on all functional diversity data obtained using STATISTICA 6 (StatSoft, Inc ©). Profiles of carbon substrate utilisation were statistically analysed by principal component analysis (PCA). PCA of the Biolog colour responses allow for comparison of microbial samples on the basis of differences in the pattern of sole-carbon-source utilisation. Shannon-Weaver diversity indices (diversity richness and evenness) were determined.

RESULTS AND DISCUSSION

FIELD STUDY:

Growth, Grain Yield, and Plant and Soil Analysis

The season was climatically difficult (Table 3). Good early rains were followed by unusually hot and dry conditions during the period shortly prior to tassle emergence and pollination and through February and March, the period during which grain fill took place. Theft of irrigation equipment made it difficult to ensure adequate moisture provision and, although there was no visual evidence of wilting, plots without cover (MB, T, MF & SF) almost certainly suffered more than those with full cover.

On several occasions, the benefits of wheat straw cover on surface moisture conditions were readily observable. Close to maturity and the formation of black layer it was also clear that the cover provided by canola, crambe and black oat residues had decomposed much faster than the wheat residue had. Thus, it seems likely that here, too, the moisture and temperature regime in treatments with wheat straw cover would have been more favourable during periods of stress. The possible implications of this will become clearer later in this discussion.

Table 3. Monthly rainfall received during the season.

Month					
October	**November**	**December**	**January**	**February**	**March**
mm					
81.9	**157.4**	**148.3**	**131.0**	**30.0**	**101.9**

Other general observations that warrant prior discussion include the initial visual effects of black oat (BO) and the season-long effect of the nematicide, Crop Guard (N). Shortly after emergence plants in BO plots were visually smaller than plants in other treatments and, while this effect cannot be unambiguously explained, since black oat is known to have allelopathic effects on weeds, it is conceivable that these effects also depressed early maize growth. The effect of Crop Guard (N) is more easily explained and was clearly a phytotoxic response. In spite of precautions having been taken to ensure that plants were avoided during the second application of this product, abnormal leaf growth became evident a week later. These symptoms disappeared within two to three weeks, but the plots continued to perform poorly right up until harvest.

Yield data presented in Table 4 clearly show that there were statistically significant treatment effects in the first, second and third samplings, and at harvest. These effects were particularly marked in the first sampling, when the yields of MB plots were almost double those of control plots (Fig. 4). At this stage, the yield of MB plots was, in fact, significantly superior to all other treatments. Yields of the next five best performing treatments (T, MF, SF, AN & CAN) were also significantly superior to those of the five worst performers (ECO, N, S, SW & C). Treatments BO, CR, OB and EXT were intermediate, CR and BO significantly so with respect to the five poorest performing treatments and the other two significantly superior to SW and C.