The effect of soil chemical properties and plant nutrition on the growth of banana (*Musa acuminata* L. A. Colla) plants infected with Fusarium wilt

by

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Declaration

I, the undersigned, hereby declare that the work in this thesis is my own original research, except where acknowledged. It has not at any time or in any form been submitted to any university for the purposes of obtaining a degree.

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CHAPTER 3: THE EFFECT OF N-FERTILISATION AND LIME ON THE GROWTH OF BANANA PLANTS INFECTED WITH FUSARIUM WILT IN A GREENHOUSE TRIAL

LIST OF ABBREVIATIONS

AR	-	Analytical Reagent
CFU	-	Colony Forming Units
DTPA	-	Diethylenediaminetetraacetic acid
EC	-	Electrical Conductivity
EDDHA	-	Ethylenediaminedi-*O*-hydroxyphenylacetic acid
EDTA	-	Ethylenediaminetetraacetic acid
ESP	-	Exchangeable Sodium Percentage
Foc	-	*Fusarium oxysporum* f.sp. *cubense*
KZN	-	KwaZulu-Natal
NH_4^+-N	-	Ammonium-nitrogen
NO_3^--N	-	Nitrate-nitrogen
SAR	-	Sodium Adsorption Ratio
SS	-	Soluble Sodium
SS%	-	Sum of Squares Percentage
VCG	-	Vegetative Compatibility Group

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ABSTRACT

Fusarium wilt of banana, which is caused by the soil-borne fungus *Fusarium oxysporum* f.sp. *cubense* (*Foc*), is a serious threat to continued banana production in Kiepersol (Mpumalanga), South Africa. Some farmers have lost up to 50% of the area originally planted to bananas due to this disease. Although several control strategies have been investigated internationally, no effective control strategies exist as yet. The objectives of this study were twofold. The first was to determine the effect of liming and nitrogen fertilisation on the growth of banana plants infected with *Foc*. The second was to determine the soil chemical properties of selected sites of three fields with soils that are suppressive or conducive to Fusarium wilt of banana in Kiepersol, in order to find possible differences between these soils and to provide basic data for future research. A soil pot trial and a hydroponic pot trial were conducted simultaneously. Banana plants were grown in the pots with a 2 x 3 x 3 factorial combination of pH, N-level and N-source used in both growth mediums. In the soil pot trial, the interaction of liming, N-levels and N-source applications yielded significant differences in plant growth, despite the infection of plants with Fusarium wilt. When no lime was applied, the application of low N-levels as a combination of both NO_3^- and NH_4^+ was most beneficial to both plant growth and nitrogen uptake. On the other hand, high N-levels, primarily in the form of NO_3^-, were only effective in promoting plant growth and nitrogen uptake when it was combined with liming. Liming did not significantly affect the soil pH and the observed effect thereof, in conjunction with N-application, could therefore be ascribed to the affect of the added Ca itself to the soil. In the hydroponic trial, the low pH treatments resulted in distinctly lower plant growth than the high pH treatments. Due to the constant mixing of the nutrient solutions in the hydroponic trial, less significant and conclusive results were generally obtained. The statistical analysis also showed that nitrogen source did not contribute to the observed results, compared to that of the soil pot trial. This indicates that the rhizosphere in the soil environment could play an important role in the observed results in the soil pot trial and this possibility needs to be investigated and quantified in further studies. Analysis of the three field soils yielded conclusive evidence that chemical differences may occur between suppressive and conducive soils. The chemical analysis of soil samples showed varied results between the three fields in terms of Ca, K, Mg, Na, P, NO_3^-, NH_4^+ and pH(H_2O), while the levels of Cu, Fe, Mn and Zn in the 0-30mmsoil layer were higher in the suppressive soils compared to the conducive soils in all three fields. Overall, results indicated that the effective management of soil chemical properties and fertilisation may aid in the manipulation of Fusarium wilt of banana in the

field. These results justify further field research in order to develop an integrated management strategy for the control of Fusarium wilt of banana and also serve as a basis for such research.

UITTREKSEL

Fusarium verwelking van piesangs, wat deur die grondgedraadge swam *Fusarium oxysporum* f.sp. *cubense* (*Foc*) veroorsaak word, hou 'n ernstige bedreiging vir die voortgesette produksie van piesangs in Kiepersol (Mpumalanga), Suid-Afrika, in. Sommige boere het reeds tot 50 % van die oorspronklike aangeplante area verloor. Ten spyte daarvan dat verskeie beheermaatreëls reeds internasionaal ondersoek is, bestaan daar tans geen effektiewe beheermaatreëls nie. Die doel van hierdie studie was tweeledig. Die eerste was om die effek van kalktoediening en stikstofbemesting op die groei van piesangplante wat met Fusarium verwelking geïnfekteer is, te bepaal. Die tweede was om die grondchemiese eienskappe van geselekteerde gedeeltes van drie lande met gronde wat onderdrukkend ("suppressive") of bevorderlik ("conducive") tot Fusarium verwelking is, te bepaal. Hierdie data is bepaal ten einde moontlike verskille tussen die gronde te vind en om fundamentele data vir toekomstige navorsing te voorsien. Twee kweekhuis potproewe is gelyktydig uitgevoer, die een in grond en die ander in 'n voedingsoplossing. Piesangplante is in die potte geplant en 'n 2 x 3 x 3 faktoriale kombinasie van kalk, N-vlakke en N-bron is in beide groeimediums toegedien. In die grond-potproef het die interaksie van die kalk, N-vlakke en N-bronne betekenisvolle verskille in die groei van die plante tot gevolg gehad, ten spyte van die infeksie van hierdie plante met Fusarium verwelking. Sonder die toediening van kalk het die toediening van lae N-vlakke as 'n kombinasie van NO_3^- en NH_4^+ die beste plantgroei, asook N-opname deur die plante tot gevolg gehad. Wanneer kalk egter toegedien is, het hoë N-vlakke hoofsaaklik in die vorm van NO_3^- die beste resultate gelewer. Kalktoediening het nie die pH van die grond betekenisvol beïnvloed nie en die effek van bekalking in kombinasie met N-toediening mag dus toegeskryf kan word aan die effek van die toegediende Ca self. In die waterkultuurproef het die laer pH-behandelings tot opvallend laer plantgroei gelei in vergelyking met die hoër pH behandelings. Die konstante vermenging van die voedingsoplossing soos veroorsaak deur lugborrels wat deur die oplossing gepomp is, het daartoe gelei dat minder betekenisvolle en oortuigende verskille in hierdie proef verkry is. Die statistiese ontleding het ook aangedui dat die bron van stikstof nie werklik 'n bydrae gelewer het tot die opgelewerde resultate nie. Dit dui daarop dat die risosfeer in die grondomgewing 'n belangrike rol kan speel in die resultate wat in die grond-potproef verkry is. Die moontlikheid hiervan moet in verdere studies ondersoek en gekwantifiseer word. Die ontleding van die drie lande het voldoende bewys gelewer dat chemiese verskille tussen onderdrukkende en bevorderlike gronde mag voorkom. Die chemiese ontledings van die grondmonsters het gevarieer tussen die drie lande in terme

van Ca, K, Mg, Na, P, NO_3^-, NH_4^+ en pH(H_2O), terwyl die vlakke van Cu, Fe, Mn en Zn in die 0-300 mm grondlaag in al drie lande hoër in die onderdrukkende as in die bevorderlike gronde was. In die geheel is dui die resultate daarop dat die effektiewe bestuur van grondchemiese eienskappe en bemesting kan help met die manupilasie van Fusarium verwelking van piesangs in die veld. Hierdie resultate regverdig verdere veldstudies ten einde ’n geïntegreerde bestuursstrategie vir die beheer van Fusarium verwelking van piesangs daar te stel en dien ook as ’n basis vir sodanige navorsing.

CHAPTER 1

INTRODUCTION

The most compelling problem that the South African banana industry is currently facing in terms of diseases is Fusarium wilt of banana, or Panama disease, as it is often called. This disease is caused by the soil-borne fungus *Fusarium oxysporum* f.sp. *cubense* (*Foc*), which can survive in the soil for many years in the form of chlamydospores (Moore *et al.*, 1999). The pathogen penetrates the plant mostly through wounds in the lateral or feeder roots and moves into the xylem where it grows and sporulates to form microconidia. The pathogen eventually prevents the upward movement of water in the plant and leads to wilting and eventual death (Jeger *et al.*, 1995).

Fusarium wilt occurs in many of the banana growing areas in South Africa, despite the control measures available to prevent the spread of the fungus and the disease. Kwazulu-Natal (KZN), Kiepersol and Komatipoort (Mpumalanga) and Tzaneen (Limpopo Province) are the areas that are currently affected (Grimbeeck *et al.*, 2001). Kiepersol, however, has been affected the worst and this research was conducted in this area in an attempt to find a viable control strategy for Fusarium wilt of banana.

Numerous studies have been conducted on Fusarium wilt around the world and many control strategies have been investigated. In terms of cultural control, these measures concentrate on preventing the spread of the disease, rather than preventing disease incidence in plants in already infested soils. Disease spread occurs mainly through the movement of infected plant material (Jeger *et al.*, 1995). Diseased areas therefore need to be isolated and plant material from these areas should not be moved to or planted in disease-free areas. Diseased areas can be 'sealed off' with a trench, which prevents the movement of the fungal spores out of the diseased area by means of surface run-off water (Deacon, 1984).

Other control measures involve good crop hygiene and farming practices. Even in areas where Fusarium wilt occurs, general crop hygiene can help reduce disease incidence and prolong the economic life of the crop (Jeger *et al.*, 1995). Healthy planting material (trimmed and treated suckers or tissue culture plants) must be used and tools, vehicle tyres and footwear need to be kept clean and fungus-free (Deacon, 1984; Smith, 2001).

Cultural control in areas where the pathogen and disease already occur, involves good general agronomic practices to ensure the optimum expression of plant resistance and to slow down but not eradicate disease spread (Stover, 1990; Jeger *et al.*, 1995). These include providing adequate plant nutrition (manipulate soil fertility to decrease growth, sporulation and virulence of the pathogen), maintaining optimum plant density, and liming (to raise the soil pH to 7.0 – 7.5 which would decrease the virulence of the pathogen) (Smith, 2001). A reduction of soil-borne inoculum by soil treatment and crop rotation, as well as flood fallowing has also been found to be effective control measures (Stover, 1962). Flooding of soil has been successful in substantially reducing subsequent wilt incidence when the soil was flooded up to at least 300 mm over four months. It also took the disease five years to reach an incidence of 50% in new plantings planted in this soil (Jeger *et al.*, 1995). Fumigants such as Vapam (methyl isothiocyanate), ethylene dibromide and formaldehyde have been tested, but were not successful enough in controlling Fusarium wilt under field conditions to be economically viable (Jeger *et al.*, 1995; Smith, 2001). In general, cultural practices are not an economical option to the control of Fusarium wilt of banana when tolerant cultivars could be used instead (Jeger *et al.*, 1995). However, in South Africa, Cavendish and Williams are the only commercial cultivars that are currently planted and no tolerant cultivars are available as yet (Fraser *et al.*, 1999). Despite the existence of control measures as described above and the awareness of farmers about such measures, Fusarium wilt is still spreading to uninfected areas, both on farms and between different areas (Grimbeeck *et al.*, 2001).

The concept of disease suppressive soils and their possible use in disease control was introduced in Central America in the 1930s (Stover, 1962). Since then, many examples of suppressive soils to various Fusarium wilt diseases have been identified and studied. Aspects such as soil pH, clay content, water content, organic matter, nitrogen source, Fe-content and more have been related to the suppressive properties of such soils (Huber & Watson, 1974; Woltz & Jones, 1981; Scher & Baker, 1982; Amir & Alabouvette, 1993; Domínguez *et al.*, 1995; Domínguez *et al.*, 1996; Peng *et al.*, 1999; Domínguez *et al.*, 2001). The possibility of biological control has also been investigated under suppressive soils, since the microbiological make-up of the soil is part of the total soil system and is influenced by both the soil physical and chemical properties. Such studies include the investigation into the effect of competition between the Fusarium wilt pathogen and other microorganisms (Louvet *et al.*, 1981; Scher & Baker, 1982; Mandeel & Baker, 1991; Toyota *et al.*, 1995). Neither a solution to Fusarium wilt of banana, nor a method in which suppressive soils can be used to eradicate

the disease, has been found yet. The concept of suppressive soils is not a static property and a change in one parameter results in changes in the other soil properties (Deacon, 1984).

More research is needed to find a solution to control Fusarium wilt and to reduce or prevent the infection of susceptible plants in fields that already contain the pathogen. Such research has been done for many years, but a concrete solution has not yet been found. It is suggested that a solution should be a multi-disciplinary approach that involves the use of plant tolerance, supported by effective suppressive soils that include a favourable microbial population, optimal growing conditions and optimal fertilisation practices. Literature on chemical and physical aspects of suppressive soils to Fusarium wilt of banana is very limited, while information on the biological aspects is more abundant. A further limitation in the current literature is the lack of field experiments that have been done. Studies are limited to the analysis of field soils as well as greenhouse trials. So far, greenhouse studies have not been repeated under field conditions and, therefore, results cannot necessarily be extrapolated to the field for advisory purposes. The research that has been done was necessary, however, because the soil conditions in the area where the disease occurs, need to be fully understood and forms the basis for further research. The same applies to greenhouse trials where the number of variables can be reduced and a specific aspect of the system of disease suppression can be investigated.

Considering the wide range of aspects that can be investigated as well as the limitations in current literature, it is not practical to investigate all aspects related to Fusarium wilt and its control through the manipulation of soil characteristics. The ideal approach would be to identify specific focus points, investigate them under controlled (greenhouse) conditions, and then test the results from such studies in the field in the specific area where Fusarium wilt problems are experienced. This includes the identification of soil characteristics, unique to the specific area, to determine the basic characteristics of the soil in question.

With regard to Fusarium wilt of banana in South Africa, few reports are currently available on soil and nutrient related topics. It is therefore difficult to ascertain the current status of soil research on this topic. What are available, however, are observations by farmers, and to a certain extent research workers, on aspects related to Fusarium wilt of banana in the field. Some farmers have identified fields or sections in fields where soils appear to be either conducive or suppressive to Fusarium wilt of banana (Hearne, *et al.*, - Personal

communication, 2002). Certain areas containing infected plants have been isolated by digging a trench around these plants and restricting access with a makeshift fence. Some farmers found that some of the isolated plants recover to a certain extent after a year or two and even produce bunches without any additional fertiliser applications (Hearne - Personal communication, 2002). Farmers generally practice intensive fertilisation of banana plants and the main nutrient of concern is N. Farmers generally apply 20 g of LAN with 20 g KCl every 2 weeks above ground per mat in a 200 - 300 mm radius until the end of autumn (Van der Walt - Personal communication, 2002). No soil test for fertiliser recommendation purposes includes a determination of N and it is therefore not known to what extent N accumulates in the soils due to these intensive fertilisation practices.

In order to study the soil aspects possibly relating to Fusarium wilt of banana in Kiepersol, it is essential that an initial study include the chemical analysis of soils in the area which show possible suppressive and conducive tendencies. In terms of plant nutritional aspects, on the other hand, greenhouse trials need to be conducted in order to eliminate as many variables as possible and focus on the effect of chosen nutrients on Fusarium wilt development and plant growth. The results obtained in such preliminary studies should then be tested under field conditions.

AIM OF THE STUDY

In the light of available research in other areas on the influence of plant nutrition on the incidence of Fusarium wilt of banana, and the current lack of information in the Kiepersol (South Africa) area, it was decided to conduct preliminary investigations. These investigations were focused on:

1. Determining the influence of the addition of lime on the incidence of Fusarium wilt of banana in greenhouse pot trials
2. Determining the influence of the level, as well as the source of N applied in greenhouse pot trials
3. Determining the differences in selected soil chemical properties between suppressive and conducive areas of three different banana fields in Kiepersol, South Africa.

The aims of the study will be addressed through a literature review (Chapter 2), greenhouse pot trials (Chapter 3), an exploratory field study and soil analysis (Chapter 4) and general conclusions and recommendations will be given (Chapter 5). The greenhouse trials were conducted simultaneously in order to create a comparison and will therefore be discussed together. For the sake of conciseness, this will be split into two chapters, one covering the Materials and Methods and the other covering the Results and Discussion. The field study will be discussed in one chapter.

The intention was that the investigations set out in the following chapters should serve as a foundation from which to launch further investigations in the field. Such investigations would focus on the influence of plant nutrition and fertilisation on the incidence of Fusarium wilt of banana in the Kiepersol area.

CHAPTER 2

THE EFFECT OF PLANT NUTRITION AND SOIL PROPERTIES ON FUSARIUM WILT OF BANANA

INTRODUCTION

Fusarium wilt diseases are considered one of the economically most important groups of diseases of agricultural crops (Engelhard, 1989). Fusarium wilt is caused by the soil-borne pathogen *Fusarium oxysporum*, a fungis that causes disease of at least 120 different crops (Hawksworth *et al*., 1995). One of these diseases is Fusarium wilt of banana (Panama disease), which is threatening continued banana production worldwide (Moore *et al.*, 1999). The first incidence of Fusarium wilt of banana was recorded in Australia in 1874, although the term Panama disease arose from early reports of the disease in the Central American region around early 1900. Before the 1960's Fusarium wilt almost destroyed the banana export industry in Central America, which was saved only by the introduction of Cavendish bananas to replace the susceptible Gros Michel bananas in the region (Stover, 1962). Cavendish bananas now also succumb to Fusarium wilt in other parts of the world. Fusarium wilt has been recorded in practically all the banana-growing areas in the world, except for those bordering the Mediterranean (Jeger *et al.,* 1995).

Several control strategies have been investigated to reduce and even eliminate the effect of Fusarium wilt on bananas (Ploetz & Pegg, 2000). These include the application of disease resistance (resistant varieties and somaclonal variants), as well as chemical-, biological- and cultural control measures. Many strategies are included under the topic of cultural control, but these measures mostly concentrate on preventing the spread of the pathogen, rather than preventing plant infections in soils that are already infected with the pathogen (Jeger *et al.*, 1995). Quarantine and exclusion practices are used to isolate diseased areas, for example, with a trench to prevent the movement of fungal spores out of this area by means of surface run-off water (Deacon, 1984). Good crop hygiene and farming practices also help to prolong the economic life of the crop in areas where Fusarium wilt occurs. These involve the use of healthy planting material and the cleaning of vehicle tyres, implements, tools and footwear to keep them fungus-free (Deacon, 1984).

The epidemic spread of plant diseases is influenced by many components of the natural environment. The soil environment directly or indirectly influences processes such as survival, dispersal, germination, host infection, growth within and outside the host and reproduction of pathogens. The host plants are also affected by the soil in terms of tissue growth, three-dimensional arrangement, physiological hardiness and disease proneness of the plants (MacDonald, 1994). Soil is a complex system that consists of air, water, mineral matter and organic matter (Brady & Weil, 1999). All four of these components undergo continuous and often simultaneous, interrelated changes that can have important effects in root disease epidemiology.

Not only is nutrient manipulation by amendment or modification of the soil environment an integral part of production agriculture, but it is also an important cultural control measure for Fusarium wilt (Stover, 1990). Although not always recognised, nutrition has always played a primary role in disease control (Huber, 1996). General agronomic practices can be employed to ensure the optimum expression of plant resistance and slow down but not eradicate disease spread (Stover, 1990; Jeger *et al.*, 1995). These include providing adequate plant nutrition through the manipulation of soil fertility to decrease growth, sporulation and virulence of the pathogen (Huber, 1996), the maintenance of optimum plant density, and to decrease the virulence of the pathogen through liming to soil pH levels of 7.0 – 7.5 (Woltz & Jones, 1981). The effect of nutrition on disease incidence can be due to an effect on either the plant or the pathogen. The effect on plant nutrition can be a direct abiotic effect caused by an added nutrient itself, or a biotic effect due to the pathogen causing either a nutrient deficiency or excess. The pathogen can, therefore, cause changes in the uptake, translocation and distribution of nutrients by the plant. In the case of Fusarium wilt, the processes that are most affected are those of translocation and distribution of nutrients in the plants (Huber, 1996).

The objective of this review is to summarise the literature available on control of Fusarium wilt by means of various soil manipulations. In the first part a brief background on Fusarium wilt of banana, the nature of the pathogen, and its mode of infection will be given. The second part of the review will focus on the South African banana industry and spreading of Fusarium wilt in the country, despite the existence of recommended quarantine measures. The role of plant nutrition in the management of Fusarium wilt will then be addressed, followed by the discussion of suppressive soils and their chemical, physical and biological characteristics.

FUSARIUM WILT OF BANANA

THE PATHOGEN

Fusarium wilt of banana is caused by the soil-borne fungus *F. oxysporum* f.sp. *cubense* (*Foc*). Once introduced into a banana field, the fungus can survive for many years as chlamydospores in previously colonised host tissues or as a parasite of weed hosts (Stover, 1962; Griffin, 1981). Initial infection of a field with *Foc* can therefore render it unsuitable for banana cultivation for more than 30 years (Stover, 1962).

The lifecycle of *F. oxysporum* is characterised by formation and dormancy of the fungus, which is followed by the germination of chlamydospores (Schippers & Van Eck, 1981). Chlamydospore formation generally takes place when carbon sources are limited and depends on the nutrient status of the inoculum. The nutrient status of the inoculum apparently determines whether deprivation or addition of nutrients stimulates chlamydospore formation. Chlamydospore survival in the soil depends on soil characteristics, climatic factors, and competition with other microbes and their ability to survive varies greatly among species and *formae speciales* (Schippers & Van Eck, 1981). Chlamydospore germination is a fairly rapid process and germination generally occurs by only one germ tube (Griffin, 1981). In nature, chlamydospore germination seems to be always dependent on external energy sources. At high spore density, chlamydospores can utilise many organic carbon sources for partial or complete germination. These include sugars, alcohols, organic acids and amino acids. Of these carbon sources, alcohols such as ethanol, and several sugars are very effective, while organic acids appear to be a less effective source (Griffin, 1981). After successful infection of the host tissue has taken place, the fungal hyphae produce micro- and macroconidia, resulting in wilting of the plants (Agrios, 1997). After the plant dies, chlamydospores will once again be formed in hyphae in the infected and decaying host tissue, but may also be formed from macroconidia that originate from sporodochia on lesions at the soil level (Christou & Snyder, 1962). A temporary supply of nutrients may also stimulate existing chlamydospores to germinate in soil, leading to the formation of chlamydospores in the germ tubes. This germination is short-lived, but can be long enough to ensure the formation of replacement chlamydospores (Kraft *et al*., 1974).

The optimum temperature for chlamydospore germination is generally 25 to 28 ºC (Byther, 1965), while little or no germination appears to take place above 37 ºC (Griffin, 1981). However, Peng *et al.* (1999) found that chlamydospore germination reached a maximum at about 30ºC and very little or no germination occurred at very low (4ºC) and very high (40ºC) temperatures. In the same study by Peng *et al.* (1999), soil water content did not significantly influence chlamydospore germination over a range of -11.5 MPa to -0.01 MPa. This is supported by the fact that *Fusaria* appear to have tolerance over a wide range of O_2 concentrations (Griffin, 1981). *Fusarium oxysporum* can grow at O_2 concentrations as low as 0.01% and *Foc* can grow under anaerobic conditions in the presence of yeast extract, MnO_2, nitrate, selenite, or ferric ions (Gunner & Alexander, 1964).

Foc can be divided into four races according to its pathogenicity to different *Musa* cultivars in the field. Race 1 occurs worldwide and attacks the Gros Michel (AAA), Silk/Rastali, Lady Finger and other members of the Pome subgroup (AAB). Race 2 is also widely distributed and is pathogenic to Bluggoe (ABB) and other closely related cooking bananas. Race 3 is restricted to Central America and is pathogenic to *Heliconia* species (Jeger *et al.*, 1995; Moore *et al.*, 2001). However, some controversy exists around the inclusion of the genus *Heliconia* in this characterisation, since *Heliconia* and *Musa* belong to different families (Heliconiaceae and Musaceae, respectively). The epithet applied to a specific *forma specialis* of *F. oxysporum* usually reflects the host genus or group of plants that it attacks. It was consequently suggested that the Race 3 isolates be excluded, as forma specialis *cubense* could not be used to describe the *Heliconia*-attacking strains and because these strains differ genotypically from the *Musa* species (Moore *et al.*, 2001). Race 4 of the pathogen is responsible for Fusarium wilt in the subtropical regions of the Canary Islands, Taiwan, Australia and South Africa and it attacks Cavendish (AAA) bananas, as well as those cultivars affected by Races 1 and 2 (Jeger *et al.*, 1995; Moore *et al.*, 2001). A new 'tropical' strain of Race 4 has recently been reported from Southeast Asia and Australia (Moore *et al.*, 2001).

Despite the fact that sexual reproduction does not occur in *Foc* populations, considerable diversity is found in this *forma specialis* (Moore *et al.*, 2001). Different isolates of *Foc* can be divided into vegetative compatibility groups (VCGs). VCGs are genetically isolated groups within the fungus, which may differ in their virulence to banana cultivars. Twenty-one VCGs have been described to date for *Foc* in the world and of these, only VCG 0120 has been found in South Africa (Visser, 2004).

DISEASE DEVELOPMENT AND SYMPTOMS

Foc penetrates plant roots mostly through wounds in the small lateral or feeder roots, and a high inoculum potential is needed to initiate the disease (Jeger *et al.*, 1995). Only a few of the lateral root infections succeed in reaching the stele of the main roots and the vascular tissues of the rhizome (Jeger *et al.*, 1995). Once *Foc* has penetrated the roots, it moves into the vascular system of the plant. Here *Foc* grows and sporulates to form microconidia that are conveyed upwards in the transpiration stream. When the pathogen reaches an end wall or perforation plate, which permits sap flow but screens microconidia, the upward movement of microconidia is momentarily halted. However, the microconidia germinate and the developing hyphae penetrate the porous barriers and sporulate above the obstruction. These spores again move upward in the xylem vessels to the next barrier and *Foc* is quickly distributed along the xylem vessels by this repetitive process (MacHardy & Beckman, 1981; Jeger *et al.*, 1995). The pathogen also produces toxins such as fusaric acid and pectolytic enzymes, which trigger the production of gels and tyloses by the plant to block off infected xylem elements. These processes, together with the breakdown of the vascular tissue as the pathogen invades the xylem parenchyma, restrict the upward transport of water in the xylem (Jeger *et al.*, 1995).

When an infected plant is cut open, the xylem of the rhizome has a reddish-brown colour where the pathogen has colonised. The first external signs of Fusarium wilt are generally seen when the leaves yellow prematurely, starting with the oldest leaves and gradually progressing to the younger leaves. Leaves eventually wilt and collapse around the pseudostem, and infection thus leads to wilting and eventual death of the plant (Ploetz, 1997). In South Africa, Fusarium wilt generally manifests itself during the winter, with the first signs of disease occurring at the start of spring. Infection then progresses throughout spring, leading to a peak disease incidence during late spring and early summer (Viljoen *et al.*, 2002).

FUSARIUM WILT OF BANANA IN SOUTH AFRICA

The early history of banana production in South Africa has not been well documented. It seems, however, that the first commercial bananas in South Africa were planted in Kwazulu-Natal (KZN), while commercial banana production in Mpumalanga and the Northern Province only started early in the 1950's (Viljoen, 2002). Currently, bananas are produced commercially in six main areas, namely Onderberg, Kiepersol, Southern-Natal, Northern-

Natal, Letaba and Levubu (Kruger *et al.*, 2002). Commercial banana production comprises of the triploid AAA Cavendish cultivars only. Initially, only the Dwarf Cavendish cultivar was planted commercially, but the Williams cultivar was introduced in 1974 (Fraser *et al.*, 1999). Until recently, South African producers have only been supplying to the local markets and no bananas were exported. However, research is currently under way to investigate export possibilities, especially to the Middle Eastern markets (Kruger *et al.*, 2002).

Due to the poor documentation of the beginning of the South African banana industry, it is not certain when and how *Foc* entered South Africa. It is possible that the disease started in KZN in the early 1900's, but no reports of the disease are available for this period. The first reported case of Fusarium wilt of banana in KZN was in 1940 (Ploetz *et al.*, 1990). The pathogen could have entered South Africa in infected banana plant material, since this, together with water draining off infected areas, is its primary means of spread. *Foc* is a highly specialised fungus for bananas and is not present in virgin soils (Deacon, 1984).

Fusarium wilt in Mpumalanga probably originated from infected banana plant material from the KZN region and possibly on a smaller scale from Mozambique (Viljoen, 2002). The presence of Panama disease in Kiepersol (Mpumalanga) dates back to at least 1970. From there, the disease spread rapidly and was well under way when the Williams cultivar was released in 1974. Although the initial Williams plants were disease-free nursery plants, farmers began to use suckers from their own diseased fields for replanting, which spread the disease throughout their farms (Deacon, 1984). The most conscientious growers in Mpumalanga were affected the worst by Panama disease, since their greater movement of workers and machinery in well-kept plantations probably enhanced the spread of *Foc* in their fields. Large areas have been forced out of production in the Kiepersol area and some growers have lost up to 50% of the area originally planted to bananas (Viljoen, 2002).

The spread of Fusarium wilt in KZN is slower than in the Kiepersol area. Production drops off within two years after planting and the replanting of plantations is needed every 3-4 years if production is to be maintained (Deacon, 1984). This is not possible in the Kiepersol area, since the spread of the disease is more rapid and the replanting of infested areas is not feasible. Lighter soils, less intensive production practices and warmer winters are all possible reasons for the slower spread of Fusarium wilt in KZN (Ploetz *et al.*, 1990). It was initially believed that four out of South Africa's six banana-growing areas could be kept disease-free.

The use of tissue culture for new plantings, as well as the geographical separation of these areas led to this assumption. Each area was also large enough to supply its own planting material and legislation exists in South Africa that controls the movement of planting material (Viljoen, 2002). Despite all these measures, the disease was introduced into the initially disease-free Tzaneen and Komatipoort areas (Grimbeeck *et al.*, 2001).

The Banana Growers Association of South Africa (BGASA) continuously receives notice of producers leaving the industry or reducing their plantings of bananas. This is, among other reasons, due to the impact of Fusarium wilt of banana on local production (Markneigings in die Piesangbedryf, 2002). In Kiepersol, Fusarium wilt has caused losses of up to 30% of banana fields since 1970 (Viljoen, 2002). In July 1998, approximately 2 500 ha were still planted to bananas in Kiepersol, of which almost 1 000 ha is threatened to be lost to Panama disease (Market Statistics, 1999). Despite several studies on the control of Fusarium wilt of banana, no management strategy has been formulated other than the use of disease resistant varieties. None of the non-Cavendish banana varieties with resistance to *Foc* Race 4 is acceptable to the local market (Viljoen, 1999). A reduction of soil-borne inoculum by soil treatment and crop rotation, as well as flood fallowing, has been found to be only temporarily effective (Stover, 1962). Currently the only effective means of controlling Fusarium wilt in South Africa is by preventing its introduction into disease-free fields (Viljoen, 2002).

COMPOSITION OF THE SOIL ENVIRONMENT

The mineral and organic particles that comprise the solid phase of soil forms a three-dimensional network, called the ‘soil matrix’ (MacDonald, 1994). Since soil particles are irregular in size and shape, considerable amounts of pore spaces occur within the soil matrix. The matrix also has a higher level of organisation or ‘structure’ due to aggregation of individual particles into larger units. This soil structure affects virtually every other property of the soil system and has many important direct and indirect effects on root disease epidemiology (MacDonald, 1994).

Air and water components coexist within the pores in the soil matrix (MacDonald, 1994). The soil water serves as a solvent, an ion transport medium, a chemically reactive substance, a physical force reshaping the solid soil matrix, and a lubricant. It is referred to as the ‘soil solution’ and its properties differ greatly from that of pure water. The soil solution properties

also differ from soil to soil, mainly as a result of complex interactions between the solid and liquid phases (MacDonald, 1994). Such interactions include cation and anion exchange, ion dissolution and precipitation, hydrogen and covalent bonding, and surface chelation (Brady & Weil, 1999). The soil solution influences many soil processes and also greatly affects root disease epidemiology. These processes include the availability of water to plant roots and microorganisms in the soil and the diffusion of chemical substances such as nutrient ions and root exudates through the soil matrix (MacDonald, 1994). Water also plays an important role in many chemical reactions that occur at the surface of soil mineral and organic particles. These reactions largely determine the availability of mineral nutrients to plants and microorganisms in the soil. The abundance or deficiency of certain macro- and micro nutrients influences the occurrence or severity of numerous root diseases (Engelhard, 1989). Soil air is a more dynamic and complex mixture of gases than the aboveground atmosphere. It mainly influences root disease epidemiology through the availability of oxygen to plant roots and microorganisms in the soil (MacDonald, 1994).

The soil rhizosphere consists of the few millimetres of soil surrounding the plant roots and its properties are influenced by the presence of the roots and their activities. The boundary between the rhizosphere and bulk soil is difficult to establish, but generally, roots affect the soil within 1 or 2 mm of the root surface. This distance can be extended to a 10 to 20 mm in the presence of mycorhizas (Wild, 1993). The inner boundary of the rhizosphere, in turn, is not necessarily sharply defined by the root surface. During root growth, the surface tissues and even the whole cortex may die and this dead material and the intercellular spaces may then be colonised by soil microbes, thereby extending the inner boundary of the rhizosphere. The rhizosphere is, therefore, not a static part of the soil. Small soil zones are continuously passing through the sequence of rhizosphere development, as influenced by the growth, development and death of roots (Tinker & Barraclough, 1988). Soil characteristics, properties of the root system, nutritional status of the plant, and climatic conditions are all factors that can influence the dimension of the rhizosphere (Lombi *et al.*, 2001).

The chemical properties of the rhizosphere are greatly influenced by root exudates. The pH in the rhizosphere can differ greatly from the pH of the bulk soil. This is mainly due to the influence of unequal uptake of cations and anions by plant roots. A dominant factor in this regard is the form of N that is taken up by plant roots, as different N-sources can result in pH differences of up to 2.2 pH units. The large difference that may exist between rhizosphere and

bulk soil pH can cause processes of adsorption or desorption, and precipitation or solubilisation of trace elements to occur. Heavy metals, for example, become more mobile in acidic conditions (Lombi *et al.*, 2001). Roots can also excrete compounds with chelating properties and can consequently affect the solubility, diffusibility and availability of trace metals such as Cu, Zn, Mn and Fe in the rhizosphere. Complexation of these elements will decrease their activities in the soil solution, thereby reducing their availability to plants and soil organisms (Tinker & Barraclough, 1988).

Microorganisms continuously break down root material. This breakdown of organic material results in the formation of humus, which is a stable part of soil organic matter (Tinker & Barraclough, 1988). The humus is environmentally very important, since it aids in the aggregation processes in soil (Brady & Weil, 1999), thereby creating functional soil that has adequate biological activity, nutrient supply and structural stability (Tinker & Barraclough, 1988). Significant amounts of organic substances are released from root surfaces. These include organic compounds with a low molecular weight such as organic acids, sugars, amino acids and phenolic compounds. Also included are mucilages with high molecular weights that provide an ideal environment for the growth of rhizosphere microorganisms (Brady & Weil, 1999). The concentration of microbes in the rhizosphere can be up to ten times that of the soil matrix, due to the additional supplies of organic substrates and specific growth factors supplied by the plant roots (Tinker & Barraclough, 1988; Brady & Weil, 1999).

THE EFFECT OF SOIL PROPERTIES ON FUSARIUM WILT

The influence of soil chemical properties on Fusarium wilt diseases has been thoroughly investigated (Huber & Watson, 1974; Woltz & Jones, 1981; Duskova & Prokinova, 1989; Jones *et al.,*1989; Domínguez *et al.*, 1996; Peng *et al.*, 1999; Domínguez *et al.*, 2001). Micro- and macro-element nutrition has been useful in the control of Fusarium wilt diseases in many crops (Table 2.1). Nutrition influences all parts of disease development through improved plant resistance, disease escape, altered pathogenicity, or microbial interactions influencing these aspects. However, effective nutrition can also be altered by the pathogen through altered uptake, translocation and distribution of nutrients in the plant (Huber, 1996).

Fusarium oxysporum is an autotroph and requires only carbon as a source of energy and structure. The essential nutrient elements needed by *F. oxysporum* include carbon, hydrogen,

oxygen, nitrogen, phosphorous, potassium, magnesium, sulphur, iron, manganese, molybdenum and zinc (Steinberg, 1950). Apart from exogenous carbon and nitrogen substrates, a balanced medium of inorganic salts is necessary for effective germination in a natural environment. *Fusarium oxysporum* has a highly developed versatility in utilisation of compounds, permitting growth and survival under many chemical-physical environments. It is able to readily undergo enzymatic adaptation, which facilitates growth and pathogenicity (Woltz & Jones, 1981).

TABLE 2.1. The effect of various plant nutrients on Fusarium wilt diseases and pathogens as found by previous researchers

Nutrient	Effect on Disease	References
NO_3^-	Increasing NO_3^- application decreased disease development	Huber & Watson, 1974; Woltz & Jones, 1981; Jones *et al.,* 1989
NH_4^+	Increasing NH_4^+ increased disease development	Woltz & Jones, 1981; Domínguez *et al.*, 1996
Soil pH	pH near 7 least optimal for Fusarium wilt; Soil pH higher in suppressive soils; Higher pH reduced number of plant infections	Woltz & Jones, 1981; Domínguez *et al*., 2001; Duskova & Prokinova, 1989
Lime and Ca	Liming increased soil suppressiveness; Liming increased number of *Foc* CFUs; Liming resulted in reduced chlamydospore germination	Höper *et al.* 1995; Peng *et al.*, 1999
K	Higher in suppressive soils Added K reduced disease	Peng *et al.,* 1999; Tharp & Wadleigh, 1939
P + lime	Reduced disease incidence	Woltz & Jones, 1973
Mg	Higher in suppressive soils	Peng *et al.,* 1999
Mn, Zn	Deficiency reduced disease	Jones & Woltz, 1967, 1969
Fe	Decreased Fe availability increased soil suppressiveness and reduced chlamydospore germination Fe-DTPA significantly higher in diseased areas	Scher & Baker, 1982; Peng *et al.*, 1999 Domínguez *et al.,* 1995; Domínguez *et al.*, 1996
Na	Higher in suppressive soils	Domínguez *et al.*, 1996; Peng *et al.*, 1999; Domínguez *et al.*, 2001;

Banana plants in turn need calcium, boron, copper and chloride in addition to the elements needed by *F. oxysporum* (Robinson, 1996). Of the essential nutrients for *F. oxysporum* and banana plants, nitrogen, potassium, calcium, magnesium and iron have been shown to affect Fusarium wilt development in soil (Table 2.1). When less than adequate amounts of nutrients such as phosphorous, magnesium, sulphur, iron, manganese, zinc and possibly copper occur in the soil, *Fusarium* propagules are less likely to establish significant inoculum levels (Woltz & Jones, 1981). In bananas on the other hand, such nutrient deficiencies could lead to several symptoms in the banana plant. These include reduced growth, small fruit, stunting, and small and horizontal bunches, depending on the specific nutrient that is deficient (Stover & Simmonds, 1987).

EFFECT OF MACRO NUTRIENTS

The most prominent studies on the effect of plant nutrition on Fusarium wilt diseases are centered around the effect of nitrogen application on disease development. In soil, high or complete germination of chlamydospores requires an exogenous source of nitrogen, but only at high spore densities. Indigenous levels of ammonium and nitrate in the soil are therefore important to chlamydospore germination (Griffin, 1969). Exogenous inorganic nitrogen also appears to have an effect on exogenous carbon metabolism and has the apparent ability to reduce the pathogen's energy requirement for germination (Griffin, 1973).

Early studies have shown that the form of nitrogen in the soil can affect Fusarium wilt diseases. Generally, fields fertilised with NO_3^--N proved to have a reduced disease incidence when compared to those fertilised with NH_4^+-N (Byther, 1965). *Fusarium oxysporum* cultivated on NO_3^--N was shown to be less virulent than the same fresh weight of the pathogen cultures cultivated on NH_3^+-N (Woltz & Jones, 1981). While Fusarium wilt pathogens are able to utilise NO_3^--N, higher levels of applied NO_3^--N often resulted in reduced disease severity (Huber & Watson, 1974). Analysis of field soils showed that diseased areas contained a higher amount of exchangeable NH_4^+ than non-diseased areas (Domínguez *et al.*, 1996). Woltz and Jones (1981) found that soils to which NH_4^+-N was applied became more favourable to disease development as the N-rate was increased, while NO_3^--N became increasingly unfavourable to the disease with increasing rate of application. The addition of nitrogen stimulates the soil microflora and the subsequent competition for nutrients and habitat decreases the number of pathogen propagules. The addition of $CaCO_3$ and NO_3^--N

raises the soil pH and decreases the virulence of *F. oxysporum* (Engelhard, 1989). On the other hand, the addition of NH_4^+-N decreases the soil and rhizosphere pH (Engelhard, 1989). It is possible that the effect of added lime and nitrate sources in controlling *Fusarium* is due to a lowered concentration of nutrients such as phosphorous, magnesium, sulphur and possibly copper in the soil, resulting from the increase in soil pH (Woltz & Jones, 1981).

The timing of nitrogen application is of importance in the control of fungal diseases. Palti (1981) states that excess nitrogen application at crop maturation could cause soft growth of leaves at a time when foliage is dense and humidity conditions particularly favourable to the pathogens. It could also delay maturation into seasons more favourable to the pathogen, thus giving the pathogen extra time to affect the crop. Lastly, it could prevent the crop from reaching full maturaty and development within its growing season. However, much of the research on the effects of late applications of nitrogen refers to cereals and their diseases and is not necessarily applicable to Fusarium wilt of banana.

An investigation on the influence of phosphorous (P) on Fusarium wilt showed that the addition of P to soil markedly reduced disease incidence in cotton compared to an untreated soil (Sadasivan, 1965). The application of P in combination with high lime resulted in a decrease in disease development in tomatoes, although high levels of P generally increased the severity of Fusarium wilt of tomato in pot and field trials. In these studies, supplemental applications of superphosphate above the required amount for growth resulted in an increase in the occurrence of Fusarium wilt of tomato in the field at soil pH 6.0. However, due to the lowered availability of P at higher pH levels, supplemental P applications at soil pH 7.0 or 7.5 did not increase wilt incidence (Woltz & Jones, 1973).

An increase in potassium (K) applied to a sand nutrient-culture trial led to a significant reduction in the severity of Fusarium wilt of cotton (Tharp & Wadleigh, 1939). Dick and Tisdale (1938) found the same effect when the severity of Fusarium wilt of cotton was decreased by increasing amounts of K applied in a field experiment. When P and K were applied separately to pots, Rishbeth (1957) found no effect on the amount of root infections in bananas, but an increase in infection was found when P and K were applied together. In the field, however, he was not able to influence disease incidence with P and K applications. In field soils, a low incidence of Fusarium wilt of banana correlated with much higher P levels than a high disease incidence did (Rishbeth, 1957). When NO_3^--N was used in conjunction

with low K application, the development of Fusarium wilt of tomato was increased, whereas high K application retarded disease development (Walker & Foster, 1946).

Calcium (Ca) nutrition can have a profound effect on vascular colonisation and Fusarium wilt development, although it is not yet clear how Ca works (Beckman, 1987). In a nutritional study, Jones and Woltz (1970) used hydrated lime and limestone amendments to increase soil pH initially to between 8.6 and 9.0. After 5 weeks, the pH levels of these soils had dropped to 7.5 and 8.0. The addition of these amendments reduced the incidence and severity of wilting in tomato. In another study, the severity of Fusarium wilt of tomato was decreased when the Ca concentration in a nutrient solution experiment was increased from 5 to 500 ppm (Edgington & Walker, 1958). Fusarium wilt was encouraged in soils with a Ca deficiency (Corden, 1965).

Contradicting results have been found in studies that determined the influence of liming on *F. oxysporum*. Höper *et al.* (1995) added lime to a conducive soil and wheat was grown in this soil for 6 months. The liming increased the suppressiveness of this soil to Fusarium wilt of flax when flax was subsequently grown in the same soil. They suggest that these results confirm the role of pH and Ca content in soil suppressiveness to Fusarium wilts. However, in the same trials, the addition of lime increased the number of *F. oxysporum* colony forming units (CFU) in the soil. In a study on Fusarium wilt of banana, the addition of $CaCO_3$ reduced chlamydospore germination by two thirds in a conducive soil and by half in a suppressive soil (Peng *et al.*, 1999). Smaller amounts of $CaCO_3$, which only raised the soil pH by 0.1 pH unit, were found to be the most effective. The addition of $Ca(OH)_2$ also reduced chlamydospore germination in both soils, but resulted in a pH increase of 3 pH units (Peng *et al*., 1999). $CaSO_4$ was as effective in reducing chlamydospore germination as $CaCO_3$, without an increase in soil pH, but more $CaSO_4$ was needed to achieve the same level of reduction in germination (Peng *et al.*, 1999).

When hydrated lime [$Ca(OH)_2$] and ground limestone ($CaCO_3$) was added to soil and the soil pH increased to 9.0, Jones and Woltz (1967, 1969) found that the development of Fusarium wilt of tomato was inhibited. They suggested that this inhibition was due to decreased availability of micro nutrients created by the increased pH of the soil solution (Jones & Woltz, 1967, 1969). They further suggested that the induced deficiency would decrease the growth, sporulation and virulence of the pathogen, although their micro nutrient data was variable and

inconclusive. In new trials, Jones and Woltz (1970) once again added a combination of $Ca(OH)_2$ and $CaCO_3$ to soil which resulted in a soil pH of 8.6 - 9.0. This increase in pH resulted in a decrease in the number of plants infected with Fusarium wilt, as well as a decrease in disease severity.

In the plant's resistance response to Fusarium wilt, the walls of xylem vessels must swell to form the gels that prevent the upward movement of microconidia of *F. oxysporum* (MacHardy & Beckman, 1981). According to Beckman (1969), calcium bridges must first be disrupted before these membranes can swell. The calcium bridges are presumably exposed to carboxylic acids by fluctuations in pH. The carboxylic acids can act on and remove the calcium bridges and cause membranes to swell, making them vulnerable to disintegration by pectic enzymes. However, Cooper *et al.* (1978) found that calcium pectate gels are resistant to degradation by pectic enzymes produced by *F. oxysporum* f.sp. *lycopersici*. The effect of added Ca on the incidence of Fusarium wilt can thus possibly be ascribed to its role in the alteration of vessel wall pectic substances (Edgington *et al.*, 1961). Palti (1981), however, stated that the primary effect of calcium on host/pathogen relationships could be on the composition of cell walls of the host, making them more resistant to penetration by facultative pathogens.

Effect of Micro Nutrients

The use of micro nutrients to control Fusarium wilt development is very important. *Fusarium* is more vulnerable to micro nutrient deficiencies than the host plant, since *Fusarium* can only utilise local nutrients (Woltz & Jones, 1981). Higher plants are able to extract micro nutrients through their root system and through the solubilising and transporting action of organic acids present in the roots that are excreted into the rhizosphere. A lack of micro nutrients in the soil is likely to prevent the Fusarium propagules from establishing at sufficient inoculum levels (Woltz & Jones, 1981).

Chlamydospores need iron to germinate and chlamydospore germination of *Foc* was decreased in soil when EDDHA and FeEDDHA, which are strong ligands for Fe, were added to the soil (Peng *et al.*, 1999). The addition of a combination of Fe, Mn and Zn lignosulfonates, as well as a combination of Fe and Mn lignosulfonates, did not significantly affect wilt development in tomato. However, the addition of a combination of Mn and Zn on the one hand and a combination of Fe and Zn lignosulfonates on the other hand, reversed the

effect of liming on disease incidence and the incidence and severity of wilt increased as their application rates were increased (Jones & Woltz, 1970). It is not known why certain combinations of micro nutrients encouraged wilt development and others did not. Micro nutrient supply in the soil could be an effective means of controlling *Fusarium*, since the fungus is more vulnerable to micro nutrient deficiencies than the host plants.

EFFECT OF PH

pH has been shown to influence the germination of chlamydospores of *F. oxysporum*, although germination does occur over a wide pH-range. For Fusarium wilt to occur, a soil pH of near or slightly above 7 has been widely shown to be the least optimal. Fusarium wilt is a disease associated with more acidic, sandy soils, rather than heavier soils with higher pH levels (Woltz & Jones, 1981). For example, a higher pH reduced the number of carnations infected with *F. oxysporum* f.sp. *dianthi* in greenhouse trials (Duskova & Prokinova, 1989). In the case of pepper, disease symptoms caused by *F. oxysporum* f.sp. *redolens* were less severe at pH 7.7 to 8.3 than at pH 6.4 to 7.0 (Sarhan & Sharif, 1986). On the complete contrary, Peng *et al*. (1999) found that chlamydospore germination of *Foc* was reduced at pH 4 to 6 and at pH 10, while chlamydospore germination and Fusarium wilt development in banana were highest at pH 8. They suggest that the soil, the race and the vegetative compatibility group (VCG) of *Foc* that they used could be responsible for the difference between these results and those of other researchers. The effect of soil pH on disease incidence has been suggested to be due to its effect on the availability of micro nutrients that are essential for the growth, sporulation and virulence of Fusarium wilt pathogens (Jones *et al.*, 1989). This view is supported by a study by Jones and Woltz (1970) in which the addition of micro nutrient compounds that were available at high pH, reversed the inhibition of Fusarium wilt of tomato.

The effects of nitrogen and pH on disease incidence seem to be at least partially connected. Fusarium wilt of tomato, cucumber, watermelon, carnation and other crops was reduced by fertilisation with NO_3^--N and lime (Jones *et al.,* 1989). The addition of NO_3^- and lime also reduced disease in tomato plants compared to the addition of NH_4^+ and lime (Woltz & Jones, 1981). The partial interaction between nitrogen form and pH is suggested to be due to the effect that nitrogen uptake by plant roots has on the pH of the rhizosphere. When plant roots absorb NH_4^+-N, the rhizosphere pH is reduced by the expulsion of H^+-ions by the roots. In the case of NO_3^--N uptake, the rhizosphere pH is increased through the release of OH^- and/or

HCO_3^- ions (Huber & Watson, 1974). A change in soil pH affects the solubility and, therefore, the availability of many nutrients in the soil. Mineral nutrients such as iron, manganese, zinc, and boron are available in acid soils, but are much less available in many alkaline soils (Brady & Weil, 1999). This reduced availability in alkaline soils can lead to deficiencies of these elements in both plants and microorganisms. Molybdenum, on the other hand, is highly available in alkaline soils, but much less available in acid soils (Brady & Weil, 1999). Many aspects of plant nutrition or soil fertility and their effects on Fusarium wilt development therefore result from changes in the chemical properties of the specific soil.

FUSARIUM WILT SUPPRESSIVE SOILS

Soils suppressive and conducive to Fusarium wilt of banana were first described in Central America in the 1930's (Stover, 1962). Very little research has been done on soil suppressiveness to Fusarium wilt of banana specifically, although research on Fusarium wilt of other crops is more readily available. The concept of soils that are suppressive to Fusarium wilt is very complex. Suppressive soils are defined as soils in which disease severity is reduced, despite the presence of the host, sufficient concentrations, and favourable environmental conditions (Louvet *et al*., 1981; Peng *et al*., 1999). Conducive soils, on the other hand, are soils with no ability to reduce disease severity (Peng *et al*., 1999).

An important measure of soil suppressiveness is the germination rate of chlamydospores (Peng *et al.*, 1999). Amir and Alabouvette (1993), however, indicated that there is not necessarily a correlation between a low disease incidence in suppressive soils and the destruction of the pathogen. A study on population dynamics showed that inoculum density dropped faster and to a lower concentration in a conducive than in a suppressive soil. The pathogen survives well in soils suppressive to Fusarium wilts (Scher & Baker, 1982) and conduciveness is not necessarily correlated with a high pathogen concentration (Amir & Alabouvette, 1993).

Soil suppressiveness is not attributed to any single soil factor, but is a combination and interaction of all soil factors, namely soil chemical, –physical and microbiological properties. Understanding the dynamics of suppressive soils and the interaction of the respective soil characteristics, however complex, can therefore contribute substantially to the eventual manipulation of soils in order to suppress or reduce Fusarium wilt.

SOIL CHEMICAL PROPERTIES

Under field conditions, the suppressiveness or conduciveness of soils to Fusarium wilt may depend on the edaphic properties of different soils (Domínguez *et al.*, 1996). In studies investigating the composition of suppressive and conducive soils, the cation exchange capacity (Domínguez *et al.*, 1996) and soil solution EC, Mg, K (Peng *et al.,* 1999), and pH (Domínguez *et al*., 2001) was shown to be higher in suppressive than in conducive soil samples. The amount of soluble Na was also higher in suppressive than in conducive soils (Domínguez *et al.*, 1996; Peng *et al.*, 1999; Domínguez *et al.*, 2001). Domínguez *et al.* (2003) investigated the ability of soil Na indices such as exchangeable sodium percentage (ESP), soluble sodium (SS) and sodium adsorption ratio (SAR) to predict soil conduciveness or suppressiveness to *Foc*. They found that SS and SAR were always greater in suppressive than in conducive soils, and indicated that SS and SAR can be satisfactory indices to study the influence of Na concentrations on the incidence of Fusarium wilt.

The availability of Fe in the soil has also been related to soil suppressiveness to Fusarium wilt diseases. The analysis of soil samples taken in suppressive and conducive banana field plots showed that FeDTPA (iron diethylenetriaminepentaacetate) was significantly higher in the diseased areas (Domínguez *et al.,* 1995; Domínguez *et al.*, 1996). In greenhouse trials the addition of FeEDTA (iron ethylenediaminetetraacetic acid) to a conducive soil did not induce suppressiveness to Fusarium wilt diseases. On the other hand, the addition of EDDHA (ethylenediaminedi-*O*-hydroxyphenylacetic acid) and FeEDDHA to this conducive soil increased the degree of soil suppressiveness to Fusarium wilt of flax, cucumber and radish (Scher & Baker, 1982). It also reduced chlamydospore germination of *Foc* in both a conducive (decrease from 75% to 15%) and suppressive (decrease from 51% to 12%) soil and halved disease severity in banana plants in both suppressive and conducive soils (Peng *et al.*, 1999). Iron is needed by chlamydospores during germination. EDDHA is a stronger ligand for Fe than either EDTA or DTPA and, therefore, reduces the availability of Fe in the soil. The reduced availability of soil Fe thus increased soil suppressiveness to Fusarium wilt of banana (Scher & Baker, 1982; Peng *et al.*, 1999).

SOIL PHYSICAL PROPERTIES

Soil physical factors include aspects such as particle-size, aggregate stability, soil solution properties, bulk density (compaction), soil temperature, soil water content, clay content and type of clay. The effect of these soil characteristics on the incidence of Fusarium wilt is difficult to determine. Studies mostly involve the identification of suppressive and conducive areas in the field and the comparison of the analyses of soil samples taken in these areas.

The presence of certain clay types has markedly affected the suppressiveness of some soils to Fusarium wilt diseases. For example, the addition of montmorillonite increased the degree of suppressiveness of a sandy soil previously conducive to Fusarium wilt of flax, while added talc resulted in a marked increase in disease incidence (Amir & Alabouvette, 1993). The amount of clay in the soil also affects the soil's microbial activity, since the clay contributes to the physical and chemical properties of the microbial habitat and also has a direct surface interaction with microorganisms (Coyne, 1999). In a study where wheat was grown for 6 months in soil treated with lime and clay and planted to flax afterwards, soil suppressiveness to Fusarium wilt of flax increased with a decrease in organic C content and pH (Höper *et al*., 1995). This was possibly due to competition between soil organisms for available C. In the same study it was found that, at pH 7, all clay additions (kaolinite, montmorillonite and illite) significantly increased soil suppressiveness compared with a limed soil unamended with clay. The addition of clay minerals, however, had different effects on soil suppressiveness, depending on the soil pH and duration of incubation, with the greatest increase in the level of soil suppressiveness always observed at high pH (Höper *et al*., 1995). In contrast, field studies done by Domínguez *et al*. (2001) showed no correlation between soil clay content and Fusarium wilt development or suppression. They did find however, that the mass of water-stable aggregates was higher in conducive than in suppressive soils.

The effect of soil temperature and soil water content on chlamydospore germination and disease incidence was investigated in pot trials using conducive and suppressive soils (Peng *et al.*, 1999). The soil temperature showed a parabolic relationship to chlamydospore germination. The maximum, optimum and minimum soil temperatures were the same for both the suppressive and conducive soils, but chlamydospore germination was markedly reduced in the suppressive soil. Germination was prevented at both low (4 - 15ºC) and high temperatures (35 - 40ºC) irrespective of the soil type, and the highest disease incidence in the conducive

soil was found at 34ºC. Soil water content also had a much more marked effect on disease severity in the conducive soil when compared to the suppressive soil, while chlamydospore germination seemed unaffected in both soils. Most *Fusarium* species are strong aerobes and survive best in drier soils, even in soils too dry to sustain plant growth (Cook, 1981).

SOIL BIOLOGY

The most important aspect of soil suppressiveness is the interaction between *F. oxysporum* and other soil microflora (Louvet *et al.*, 1981), with two types of suppressiveness known in terms of biological suppressiveness. The first, general suppression, is suppression due to the total microbial biomass in the soil and is not transferable between soils. The second, specific suppression, is due to the effects of individual or selected groups of microorganisms and is transferable between soils (Weller *et al.*, 2002). Other microorganisms can directly influence the pathogen by competing for available nutrients in the soil and thereby preventing the pathogen from developing. Microorganisms can also inhibit the pathogen's activities toward the host by competing for infection sites on the plant roots (Louvet *et al.*, 1981). The most important of these interacting microflora include saprophytic strains of *F. oxysporum*, other *Fusarium* spp. such as *F. solani*, bacteria, other fungi and actinomycetes (Louvet *et al.*, 1981; Jeger *et al.*, 1995).

The effect of two non-pathogenic strains of *Fusarium oxysporum*, C5 and C14, was investigated in the biocontrol of Fusarium wilt of cucumber (Mandeel and Baker, 1991). The addition of both non-pathogenic strains to raw soil reduced the germination of chlamydospores of *F. oxysporum* f.sp. *cucumerinum* in the rhizospheres of cucumber. They suggest that this reduction in germination could be due to carbon competition between the various strains. The addition of Strain C14 resulted in a decrease in plant infections and was influenced by the inoculum density of the pathogen. A significant decrease in infection occurred at population densities of Strain C14 above 8×10^4 CFU g^{-1} of soil. Strain C5, on the other hand, was not able to induce a significant decrease in infections by *F. oxysporum* f.sp. *cucumerinum*.

The addition of *Pseudomonas putida* to a conducive soil rendered the soil suppressive to Fusarium wilt of flax, cucumber and radish (Scher & Baker, 1982). The *P. putida* competed with *F. oxysporum* for available Fe in the soil, since Fe seems necessary for germ tube

elongation of *F. oxysporum* f.sp. *lini* microconidia. Toyota *et al.* (1995) found that the growth of *F. oxysporum* f.sp. *raphani* (Fusarium wilt of radish) was suppressed in soil that was previously colonised by microorganisms, especially other *Fusarium* species. They ascribe the result to competition for the available nutrients and the consequent exhaustion of nutrients by the established colonisers. Fewer nutrients were thus available for the subsequent growth of the tomato wilt pathogen. However, the addition of nutrients to these soils did not increase the growth of the tomato wilt pathogen, suggesting the continued competition for nutrients.

The addition of antagonistic bacteria to the roots of carnation cuttings consistently reduced Fusarium wilt severity in these plants (Yuen *et al.*, 1985). However, the use of individual strains of bacteria to control Fusarium wilt of carnations in commercial production is not practical. The carnations are grown for up to two years and are susceptible to the disease for the entire period. The roots are also continuously growing and moving away from areas in the soil with the highest population densities of the introduced bacteria (Yuen *et al.*, 1985).

SUMMARY AND DISCUSSION

A shortage exists in research on Fusarium wilt of banana specifically. The need also exists for more field research, especially on the effect of applied nutrients on Fusarium wilt development and plant growth. The earlier studies included more field research, but later research was more confined to greenhouse trials. The greenhouse results need to be tested in the field.

Results from different studies do not always correlate, which emphasises the need for research in each area where Fusarium wilt occurs. From the results already obtained, it is clear that nutrition, both in terms of the plant and the pathogen, plays an important role in Fusarium wilt development. The most prominent nutritional results so far are those on the effect of nitrogen fertilisation and pH on Fusarium wilt. However, the effect of micro nutrients needs to be investigated further, especially since the pathogen is more sensitive to micro nutrient deficiencies than the host plant.

Control strategies to reduce the impact of Fusarium wilt on banana have been investigated for many years. Other than the use of disease resistant varieties, no effective management strategy has been designed to eradicate or reduce the impact of Fusarium wilt in diseased banana

fields. The strategies that are available, such as quarantine, exclusion, the use of clean planting material, chemical control, biological control and sanitisation of farm implements, are either not effective, economically viable, or only prevent the dissemination of the pathogen to uninfested fields. Despite the availability of preventative measures, there is still a general lack of implementation, and Fusarium wilt is still spreading to disease-free areas. Also, the taste of disease resistant banana varieties is often unacceptable to local markets. A different approach, therefore, is needed to ensure the sustainable production of bananas in banana fields with Fusarium wilt.

Plant nutrient manipulation by amendment or modification of the soil environment is an integral part of crop production, and has proved to be an important cultural control measure for Fusarium wilt diseases of agricultural crops (Jones *et al*., 1989). For Fusarium wilt of banana, unfortunately, this has not proven to be the case. While nitrogen and its application form has shown to be an important factor in reducing Fusarium wilt in other crops, little to no work in this regard has been done on banana. In general, a pH of 7.0 to 7.5 seems the best for disease suppression in many crop systems (Smith, 2001) and is effective in conjunction with the application of nitrogen in the nitrate form (Engelhard, 1989). Yet the only work done on Fusarium wilt of banana is a report by Peng *et al.* (1999) where a complete contrary is reported.

It was proposed that the effect of added lime and nitrate was due to the lowered availability of nutrients such as phosphorous, magnesium, sulphur and possibly copper (Woltz & Jones, 1981). It was generally found that the addition of nitrate, lime, potassium, and calcium reduced the effect of Fusarium wilt on various crops. The addition of phosphorous was effective in curbing Fusarium wilt when the pH was sufficiently high to reduce the availability of phosphorous. On the other hand, a deficiency of micro nutrients was effective in reducing the effect of Fusarium wilt, since the fungus is more vulnerable to micro nutrient deficiencies than the host plants (Woltz & Jones, 1981).

It is worthy of note that much of the research on the influence of nutritional aspects on Fusarium wilt, especially under field conditions, was done several years ago. The latest research is mostly restricted to studies done in the laboratory and in pot trials. A lack of current field research therefore exists and studies should be repeated in and adapted to field conditions. It is also evident that a lot of contradictory results are found in studies conducted

by different researchers. Each area where Fusarium wilt of banana occurs needs to be evaluated separately to determine what the influence of nutrition will be on disease development in that specific area. Results should also be tested in the field, since laboratory and greenhouse results cannot be extrapolated to field conditions. This study is thus intended to form the basis of such research in South Africa and specifically in the Kiepersol (Mpumalanga) area where disease incidence is very high.

CHAPTER 3

THE EFFECT OF N-FERTILISATION AND LIME ON THE GROWTH OF BANANA PLANTS INFECTED WITH FUSARIUM WILT IN A GREENHOUSE TRIAL

INTRODUCTION

Nutrition influences all parts of disease development through improved plant resistance, disease escape, altered pathogenicity, or microbial interactions influencing these aspects (Huber, 1996). Various international studies have thus been done to determine the effect of nutrition on Fusarium wilt development in various crops. A number of these studies indicated that liming and nitrogen application can influence Fusarium wilt pathogens and disease development. (Woltz & Jones, 1981; Duskova & Prokinova, 1989; Jones *et al.,* 1989; Höper *et al.,* 1995; Peng *et al.*, 1999).

Both the level of nitrogen application, as well as the form of nitrogen applied has been shown to affect Fusarium wilt diseases and pathogens. Fields fertilised with NO_3^--N generally had a reduced disease incidence when compared to those fertilised with NH_4^+-N (Byther, 1965) and *Fusarium oxysporum* was less virulent when cultivated on NO_3^--N compared to NH_4^+-N cultivation (Woltz & Jones, 1981). Increasing levels of applied NO_3^--N was generally found to be less favourable to Fusarium wilt development (Huber & Watson, 1974; Woltz & Jones, 1981), while increasing levels of NH_4^+-N were more favourable to disease development (Woltz & Jones, 1981). Woltz and Jones (1981) suggested that nitrogen application stimulates microflora, resulting in a decrease in the number of pathogen propagules through competition for nutrients and habitat.

Liming has been shown to increase soil suppressiveness to Fusarium wilt of flax (Höper *et al*., 1995), although, in the same trials, the addition of lime increased the number of *F. oxysporum* colony forming units (CFU) in the soil. In a study on Fusarium wilt of banana, the addition of $CaCO_3$ reduced chlamydospore germination by two thirds in a conducive soil and by half in a suppressive soil (Peng *et al.,* 1999), with smaller amounts of $CaCO_3$ proving to be the most effective. The addition of hydrated lime [$Ca(OH)_2$] and ground limestone ($CaCO_3$) resulted in an a soil pH of 9 and inhibited the development of Fusarium wilt of tomato (Jones & Wotlz, 1967, 1969). This inhibition was suggested to be the result of decreased availability of micro

nutrients creasted by the increase pH of the soil solution. In further trials, Jones and Woltz (1970) found that a combination of $Ca(OH)_2$ and $CaCO_3$ added to soil, resulted in a soil pH of 8.6 - 9.0, a decrease in the number of plants infected with Fusarium wilt and a decrease in disease severity.

It has also been proposed that there is at least a partial interaction between lime and nitrogen in the soil in terms of their effects on Fusarium wilt. This interaction was suggested to be due to the rhizosphere effect of nitrogen uptake by plants, whereby the uptake of nitrogen in the form of NO_3^- or NH_4^+ could respectively increase or decrease the pH of the rhizosphere (Huber & Watson, 1974).

Based on these findings, a greenhouse trial was conducted to determine the influence of liming and nitrogen application on the growth of banana plants in the presence of *Foc*, when planted in soil obtained from Kiepersol (Mpumalanga), South Africa, where Fusarium wilt of banana occurs. This trial was expanded to include a hydroponic pot trial, with the intention of removing the rhizosphere and comparing the results with those of the soil pot trial.

MATERIALS AND METHODS

SOIL POT TRIAL

Soil Used for Greenhouse Pot Trials

Soil containing *Foc* that resulted in a high incidence of Fusarium wilt of banana in field trials was obtained from a farm in Kiepersol, Mpumalanga in South Africa, and used as growth medium for this pot trial. This soil was chosen to ensure that the soil was indeed conducive to Fusarium wilt development. To ensure that both the natural *Foc* inoculum and other natural soil microorganisms were not destroyed, the soil was not sterilised before experimentation. Each pot contained 4 kg of soil and four repetitions were included for each treatment. A sample of the soil was air-dried and analysed for certain chemical properties as follows:

Determination of Soil pH

Both the $pH(H_2O)$ and pH(KCl) were determined using a 1:2.5 extract (Non-Affiliated Soil Analysis Work Committee, 1990) where 20 g of soil and 50 ml of de-ionised water or 1 M

KCl was stirred rapidly for 5 seconds with a glass rod. The mixture was stirred again after 30 minutes, after which the pH was determined with a calibrated pH meter, with the electrodes positioned in the supernatant liquid.

Determination of Soil Phosphate

The phosphate determination was done according to the Bray-1 method where 50 ml of Bray-1 solution was added to 6.67 g of soil and was shaken manually for 60 seconds. Two drops of flocculant was added and the solution was filtered immediately through Whatman 2V filter paper. The amount of phosphate in the filtrate was determined with an Auto Analyzer (Non-Affiliated Soil Analysis Work Committee, 1990).

Determination of Soil NO_3^- and NH_4^+

The determination of both NO_3^- and NH_4^+ content in the soil was done through steam distillation (Non-Affiliated Soil Analysis Work Committee, 1990). The soil samples were oven-dried overnight at 40ºC and 100 ml of a 1 N solution of KCl was added to 50 g of the soil. The suspension was mechanically shaken for 1 hour, filtered and the N-content of the filtrate was determined.

For the determination of NH_4^+, 15 ml of a 50% (v/v) NaOH solution was added to 50 ml of the filtrate obtained above and distilled for 7 minutes into 25 ml boric acid. The distillate was titrated with 0.01 M HCl and the amount of NH_4^+ was calculated. For the determination of NO_3^-, a spatula tip of Davarda Alloy was added to the distilled sample and left to stand until the solution was fully reduced. The solution was then distilled for 7 minutes into 25 ml boric acid and the distillate was titrated with 0.01 M HCl. The amount of NO_3^- was then calculated.

Determination of Soil Ca, Mg, K and Na

The determination of Ca, Mg, K, and Na was done by extraction with a 1 M ammonium acetate solution at pH 7 (Non-Affiliated Soil Analysis Work Committee, 1990). The extraction was conducted by the addition of 50 ml of a 1 M NH_4OAc solution at pH 7 to 5 g of soil and shaking the solution at 180 oscillations per minute for 30 minutes. The solution was filtered, whereafter the Ca and Mg in the filtrate was determined by atomic absorbtion

spectorphotometry and the K and Na by flame emission spectroscopy (Non-Affiliated Soil Analysis Work Committee, 1990).

Fertiliser Treatment of the Test Soil

To determine the effect that liming (pH and Ca level) has on Fusarium wilt development, the potting soil was divided into two sets. One set was treated with lime (L), and the other set was not treated with lime (WL). The equivalent of 2.6 t ha^{-1} $CaCO_3$ (AR) (lime) (2.35 g $CaCO_3$ per pot) was added to the L treatments (Table 3.1), while no extra Ca in any form was added to the WL treatments. Each pot received the equivalent of 100 kg K ha^{-1} (0.1693 g KCl – assumed to be incorporated to a depth of 300 mm in a field soil) in the form of KCl (AR).

Three levels of nitrogen were applied, namely the equivalent of 75, 150 and 225 kg N/ha/year (7.7, 15.4 and 23.1 mg N pot^{-1} every 3 weeks). To determine the influence of the form of nitrogen applied, each nitrogen level was applied in three NO_3^-:NH_4^+ ratios of 9:6, 11:4 and 14:1, respectively. The nitrogen treatments were split into 3-weekly applications and applied as a solution to ensure that NH_4^+ was not nitrified to NO_3^-. The amounts of the stock solutions of 0.01 M NH_4NO_3 (AR) and 0.01 M KNO_3 (AR) applied are summarised in Appendix A.

Inoculation of Soil Pots with Foc

An isolate of *Foc* was grown on millet seed by placing the seed in glass bottles and soaking it in water for 24 hours. Thereafter, all excess water was drained off and the seed was autoclaved twice. The millet seed was then inoculated with an agar plug containing mycelia of *Foc*-Isolate 045 and placed in an incubator at 25 ºC for 2 weeks. The bottles were shaken manually every 3 days to ensure that the fungus was spread evenly throughout the seed. During the incubation time the lids of the bottles were screwed on loosely for aeration.

Immediately after the soil was treated with the respective fertilisers, 250 ml of *Foc*-colonised millet seed was added to each pot. One Cavendish banana tissue culture seedling, from Du Roi Laboratories, Letsitele, South Africa, was planted per pot. The pots were placed on a rotating table in a greenhouse to ensure even climatic conditions during the growth period and the plants were watered daily with de-ionised water.

TABLE 3.1 Lime and nitrogen soil treatments of banana plantlets grown in pots with 4 kg soil inoculated with *Fusarium oxysporum* f.sp. *cubense*

Treatment	Additions for the treatment
WL (Without Lime)	No $CaCO_3$ added
L (Lime)	2.35 g $CaCO_3$ (AR) added per pot
N1	$^{\uparrow}$7.7 mg N pot^{-1} every 3 weeks
N2	$^{\uparrow}$15.4 mg N pot^{-1} every 3 weeks
N3	$^{\uparrow}$23.1 mg N pot^{-1} every 3 weeks
V1	NO_3^- : NH_4^+ = 9 : 6$^{\updownarrow}$
V2	NO_3^- : NH_4^+ = 11 : 4$^{\updownarrow}$
V3	NO_3^- : NH_4^+ = 14 : 1$^{\updownarrow}$
C1 (Control)	WL, 0 kg N ha^{-1}
C2 (Control)	L, 0 kg N ha^{-1}

$^{\uparrow}$ The amount of N applied over a year would amount to 75, 150 and 225 kg N ha^{-1} yr^{-1} for N1, N2 and N3, respectively

$^{\updownarrow}$ Ratios were calculated on a $mmol_c$ basis

Harvesting and Evaluation of Banana Plants for Fusarium Wilt Development

Plants were harvested after 6 weeks of growth when the first banana plants died due to Fusarium wilt. The death of these plants indicated that sufficient time had passed to allow the infection of banana plants with *Foc* and disease development. The plants were rated on a scale of 0-5 for internal disease symptoms. The internal symptoms were rated according to the degree of discolouration of the rhizome just above the root entry level. A rating of 0 indicated no discolouration, whereas a rating of 5 indicated that the total inner rhizome was affected (discoloured). Ratings of 1 to 4 ranged from isolated spots of discouloration (rated 1) to more than two thirds of the inner rhizome being discoloured (rated 4). *Foc* was isolated from the plant material to ensure that the infection was indeed caused by the inoculated pathogen. Due to the subjectivity of the rating procedure, this data was used only as an indicator of the extent of disease incidence in the plants. In addition, the above ground material was dried at 60 ºC for 48 hours and the dry mass as well as the N-content of the plant material was determined.

Determination of Plant NH_4^+

The amount of reduced nitrogen (NH_4^+) in the above ground plant material was measured to determine the amount of nitrogen that was taken up by the plants in the presence of the Fusarium wilt pathogen. The NH_4^+-content was determined by steam distillation after an H_2SO_4 digestion as prescribed by ALASA (1998). This method determines N-content in the above ground dry material as a mass/mass %.

Statistical Analysis

The experiment was designed as a completely random design with four repelicates and 18 factorial treatment combinations. The dry mass and NH_4^+-content of the above ground material were analysed by factorial ANOVA to test for differences between the three main effects, as well as all interactions. The data was acceptably normal with homogenous treatment variances. Treatment means were separated using Fishers' protected t-test least significant difference (LSD) at the 5% level of significance (Snedecor & Cochran, 1980). Data were analysed using the statistical programme GenStat (2003).

HYDROPONIC TRIAL

The hydroponic trial was conducted in the greenhouse in containers (10L) with two banana seedlings from the Du Roi Laboratories, Letsitele, South Africa, per pot and four repetitions. The containers were placed on a rotating table to ensure even climatic conditions during the growth period. The treatments used in this trial are given in Table 3.2.

Hydroponic Growth Medium

A modified Hoagland 2 nutrient solution was used as growth medium in this trial. The amounts of stock solutions applied to each pot are given in Appendix B and C. Stock solutions with concentrations of 1 M were used for all the macro elements, except K_2SO_4, which was a 0.5 M solution and $CaSO_4 \cdot 2H_2O$, which was a 0.1 M solution. The recipes for the micro-element stock solutions are given in Table 3.3. Air was bubbled through the solution at fixed intervals to aerate and ensure mixing of the solution in order to eliminate any possible rhizosphere effect.

TABLE 3.2 Treatment of the hydroponic growth medium of banana plantlets grown in containers (10 L) and inoculated with *Fusarium oxysporum* f.sp. *cubense*

Treatment	Additions for the treatment
Low pH	Adjusted to solution pH 6
High pH	Adjusted to solution pH 7
N1	½ x Hoagland N (7.5 $mmol_c\ N\ L^{-1}$)
N2	1 x Hoagland N (15 $mmol_c\ N\ L^{-1}$)
N3	1½ x Hoagland N (22.5 $mmol_c\ N\ L^{-1}$)
V1	NO_3^- : NH_4^+ = 9 : 6[↕]
V2	NO_3^- : NH_4^+ = 11 : 4[↕]
V3	NO_3^- : NH_4^+ = 14 : 1[↕]
C Low pH (Control)	Low pH, N2, V3, No *Foc*
C High pH (Control)	High pH, N2, V3, No *Foc*

[↕] Ratios were calculated on a $mmol_c$ basis

TABLE 3.3. The amounts of each compound made up to a 1 L stock solution for the micro elements to be added to the water solution inoculated with *Fusarium oxysporum* f.sp. *cubense*

Micro element	Amount of Compound
Fe	5.99 g $FeSO_4.7H_2O$ + 4.01 g Na_2EDTA
Mn	2.28 g $MnSO_4.H_2O$
Cu	0.08 g $CuSO_4.5H_2O$
Zn	0.22 g $ZnSO_4.7H_2O$
B	2.86 g H_3BO_3
Mo	0.03 g $Na_2MoO_4.2H_2O$

Adjustment of Solution Nitrogen

Three levels of nitrogen were applied, namely 7.5, 15 and 22.5 $mmol_c\ N\ L^{-1}$. These nitrogen levels are ½, 1 and 1½ times the normal Hoagland 2 solution nitrogen levels. To determine the influence of the form of nitrogen applied, each nitrogen level was applied in three NO_3^-:NH_4^+ ratios of 9:6, 11:4 and 14:1, respectively.

Adjustment of Solution pH

Two pH treatments were included in this trial and the solution pH was increased by the addition of $KHCO_3$. The solution was adapted to accommodate the added K and was replaced every 3 weeks. The low pH and high pH treatments were adjusted to a pH of 6 and 7, respectively.

Preparation of Inoculum

An inoculum was prepared in a sporulation medium made up from the ingredients given in Table 3.4. The stock solutions were made up in Erlenmeyer flasks, closed with cotton wool and covered with foil to prevent contamination while still allowing aeration. The solutions were autoclaved and inoculated with filter paper containing micelia of *Foc* (isolate 092). The flasks were then shaken for 6 days on an automatic shaker until sufficient fungal growth had occurred. The solutions were filtered through cheesecloth to remove the micelia and the spore concentration of the solutions was determined with a haemacytometer.

TABLE 3.4. The sporulation medium used to prepare 1 L of concentrated inoculum of *Fusarium oxysporum* f.sp. *cubense* for inoculation in nutrient solutions in the hydroponic trial (distilled water was used to make up the 1 L solution)

Compound	Amount made up to 1 L
Sucrose	20 g
$MgSO_4 \cdot 7H_2O$	0.4 g
KCl	1.6 g
KH_2PO_4	1.1 g
$Ca(NO_3)_2$	5.9 g
100 mg ml^{-1} $MnSO_4$	20 µl
10 mg ml^{-1} $ZnSO_4$	20 µl
10 mg ml^{-1} $FeCl_3$	20 µl

Inoculation of the Growth Medium

Plants were grown in the nutrient solutions for 4 weeks to ensure sufficient root development, after which time the nutrient solutions were inoculated to reach a spore concentration of 8.5 x 10^4 spores *Foc* cm^{-3} in each container. None of the control treatments were inoculated with

Foc, but they received the equivalent nitrogen of the treatments N2 and V3 (Table 3.2), which are the standard Hoagland 2 solution nitrogen levels. The inoculation of the nutrient solutions took place a week after the growth medium was replaced and the solution pH was measured before inoculation with *Foc*. Since the nutrient solution was replaced every 3 weeks, the inoculum was only present in the solution for 2 weeks.

Evaluation of Plants

The plants were harvested 5 weeks after inoculation with *Foc* (9 weeks in total), when sufficient disease symptoms had developed in the plants, and were rated on a scale of 0-5 for internal disease symptoms. The internal symptoms were rated according to the degree of discolouration of the corm just above the root entry level. A rating of 0 indicated no discolouration, whereas a rating of 5 indicated that the total circumference of the vascular tissue showed discolouration. Ratings of 1 to 4 ranged from isolated spots of discolouration (rated 1) to more than two thirds of the vascular tissue being discoloured (rated 4). The above ground matter was dried at 60 ºC for 6 days and the dry mass and N-content of the material was determined. *Foc* was isolated from the plant material to ensure that the infection was caused by the inoculated pathogen.

Method of Plant Analysis

Determination of Plant NH_4^+

The amount of reduced nitrogen (NH_4^+) in the above ground plant material was measured to determine how much nitrogen was taken up by banana plants in the presence of the Fusarium wilt pathogen. The NH_4^+ was determined colorimetrically on an Auto Analyzer after an H_2SO_4 digestion as prescribed by ALASA (1998). This method determines the N-content of the above ground dry material as a mass/mass %.

Statistical Analysis

The experiment was designed as a completely random design with four repelicates and 18 factorial treatment combinations. The dry mass and NH_4^+-content of the above ground material were analysed by factorial ANOVA to test for differences between the three main effects, as well as all interactions. The data was acceptably normal with homogenous treatment variances. Treatment means were separated using Fishers' protected t-test least

significant difference (LSD) at the 5% level of significance (Snedecor & Cochran, 1980). Data were analysed using the statistical programme GenStat (2003).

RESULTS AND DISCUSSION

SOIL POT TRIAL

The soil used in this trial was already infected with *Foc*, since it was obtained from a field in Kiepersol (Mpumalanga) where Fusarium wilt of banana was known to occur. Two of the four control plants at each pH level were infected with *Foc* without the addition of extra inoculum and the control was therefore not disease-free as was the initial intention. Consequently, the control treatments were not included in the presentation of results below.

Analysis of the Soil Used as Growth Medium

Analysis of the soil used as growth medium indicated sufficient levels of P, Ca, Mg and Na to provide adequate nutrition to banana plants for the duration of this trial (Table 3.5). However, the level of K was considered too low and K-fertilisation of 0.1693 g KCl was applied per pot.

TABLE 3.5. Selected properties of soil from Kiepersol (Mpumalanga), South Africa, used as growth medium for banana plants in the greenhouse pot trial before treatment with fertilisers

Chemical Analysis	Value
pH (H_2O)	6.7
pH (KCl)	5.9
P (Bray-I)	30.2 mg kg^{-1}
KCl-extractable NO_3^-	7.88 mg NO_3^- kg^{-1}
KCl-extractable NH_4^+	1.64 mg NH_4^+ kg^{-1}
Ca (1 M NH_4OAc)	4.52 $cmol_c$ kg^{-1}
Mg (1 M NH_4OAc)	0.97 $cmol_c$ kg^{-1}
K (1 M NH_4OAc)	0.57 $cmol_c$ kg^{-1}
Na (1 M NH_4OAc)	0.12 $cmol_c$ kg^{-1}

The Effect of Lime and Nitrogen Application on Fusarium Wilt Development

Internal symptoms of Fusarium wilt developed in all banana plants inoculated with *Foc*, regardless of the treatment that was applied. No significant difference in disease incidence was found among the treatments, while an increase in disease severity from less than 1 to 4 resulted in a decrease in above ground dry mass production from 4 g to 2 g (Figure 3.1). These results were found with and without the addition of lime and differences in dry matter production were observed between different treatments despite the high infection rate.

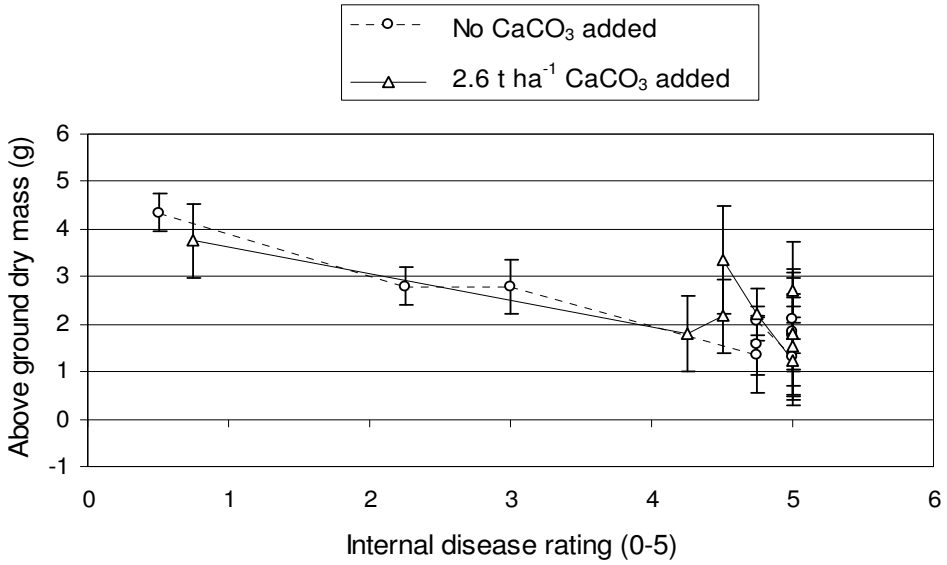

FIGURE 3.1. The internal disease rating in relation to the above ground dry mass of banana plants grown in pots with soil treated with different pH and nitrogen combinations, and inoculated with *Fusarium oxysporum* f.sp. *cubense* in the soil pot trial. The internal disease symptoms were rated according to the degree of discolouration of the rhizome just above the root entry level, with a rating of 0 for no discolouration, up to a rating of 5, indicating that the total inner rhizome was discoloured. The vertical bars indicate the standard deviations of the respective values.

The Effect of Lime and Nitrogen Application on Aboveground Dry Matter Production

Due to the high buffer capacity of the soil, the added $CaCO_3$ in the limed treatments only increased the soil pH by an average of 0.05 pH units (Table 3.6). Despite the small effect on

soil pH, liming affected the growth of the plants in some treatments (Table 3.7; Figure 3.2). The factorial ANOVA (Appendix D) showed a pH x nitrogen level interaction [Sum of Squares % (SS%) = 10.39%], although the SS% indicated that the nitrogen level x nitrogen source interaction accounted for 11.68% of the variance in the results and the pH x nitrogen level x nitrogen source interaction accounted for the third highest percentage variance of 6.09%.

TABLE 3.6. Average pH-values of the soil in pots treated with different lime and nitrogen combinations, and inoculated with *Fusarium oxysporum* f.sp. *cubense*.

Nitrogen application		pH(H_2O)	
Amount	NO_3^-:NH_4^+ ratio	Without Lime	With Lime
7.7 mg N	9:6	6.35	6.63
7.7 mg N	11:4	6.42	6.54
7.7 mg N	14:1	6.45	6.46
15.4 mg N	9:6	6.44	6.48
15.4 mg N	11:4	6.50	6.56
15.4 mg N	14:1	6.55	6.55
23.1 mg N	9:6	6.46	6.44
23.1 mg N	11:4	6.45	6.47
23.1 mg N	14:1	6.50	6.51

The effect of liming on plant growth was only significant in treatments with the lowest and the highest nitrogen applications and had an opposite effect at these two extremes, as well as with an increase in the amount of N applied in the form of NO_3^-. Liming, accompanied by the lowest N-application at a NO_3^-:$NH4^+$ ratio of 9:6 resulted in a significant (45%) decrease in above ground dry mass of plants relative to the unlimed treatment. At the other extreme, the highest nitrogen application at a NO_3^-:$NH4^+$ ratio of 14:1 in the unlimed treatment resulted in a significant (60%) decrease in the dry mass relative to the limed treatment (Figure 3.2).

TABLE 3.7. Average dry mass of the above ground banana plant material grown in pots with 4 kg soil treated with different fertiliser combinations, and inoculated with *Fusarium oxysporum* f.sp. *cubense*. Values followed by the same letter do not differ significantly (P<0.05). SEM = 0.43, LSD = 1.21, CV = 42.5%.

Nitrogen application		Dry mass (g)	
Amount	NO_3^-:NH_4^+ ratio	Without Lime	Lime
7.7 mg N	9:6	2.79^{ab}	1.53^{cd}
7.7 mg N	11:4	2.78^{ab}	2.70^{abc}
7.7 mg N	14:1	1.84^{bcd}	1.80^{bcd}
15.4 mg N	9:6	2.11^{bcd}	2.17^{abcd}
15.4 mg N	11:4	1.36^{d}	1.81^{bcd}
15.4 mg N	14:1	1.56^{cd}	2.21^{abcd}
23.1 mg N	9:6	1.77^{bcd}	1.22^{d}
23.1 mg N	11:4	2.07^{bcd}	1.81^{bcd}
23.1 mg N	14:1	1.33^{d}	3.35^{a}

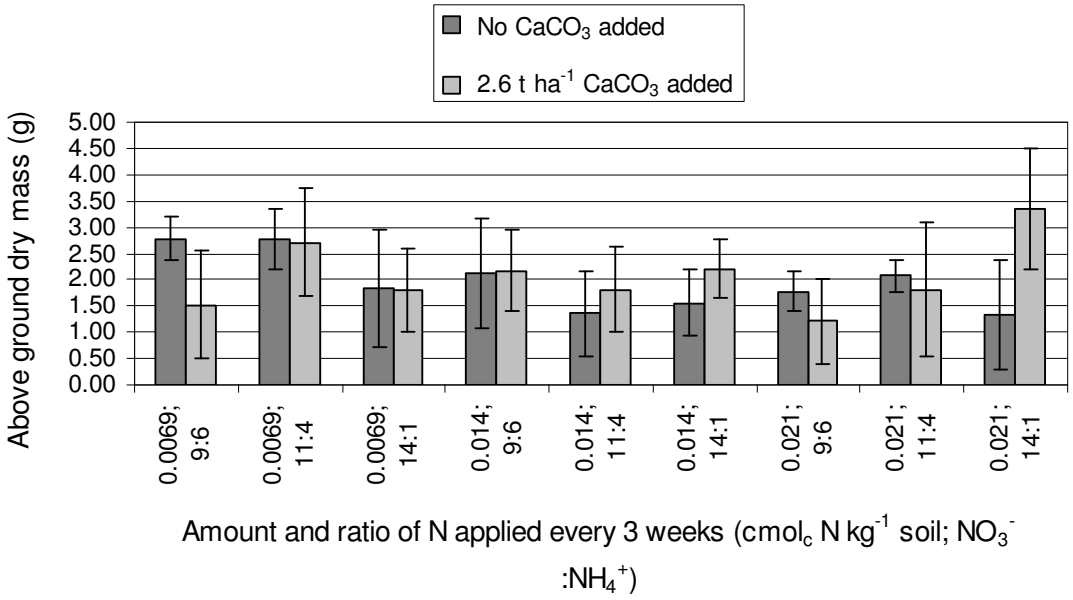

FIGURE 3.2. The above ground dry mass of banana plants grown in pots with soil with and without the addition of lime, at different nitrogen applications and inoculated with *Fusarium oxysporum* f.sp. *cubense*. The vertical bars indicate the standard deviations of the respective values. SEM = 0.43, LSD = 1.21, CV = 42.5%.

Without the addition of lime, an increase in applied nitrogen from the lowest to the highest applications resulted in a non-significant decrease in dry matter production of 36%, 25% and 27%, respectively, regardless of the NO_3^-:NH_4^+ ratio that was applied (Figure 3.3). Even though these differences were not significant, it indicates a distinct trend in plant growth in relation to increased nitrogen application. An increase in the applied NO_3^-:NH_4^+ ratio from the 9:6 to 14:1 also resulted in a non-significant decrease in dry matter production of 34%, 26% and 25%, respectively, at all three nitrogen levels applied.

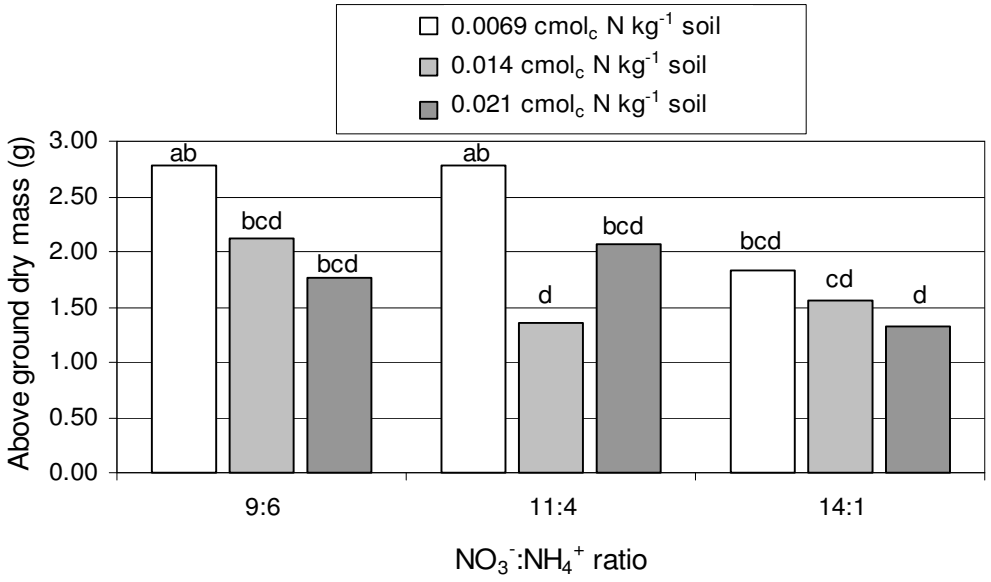

FIGURE 3.3. The effect of three N-levels applied every 3 weeks to pots with soil inoculated with *Fusarium oxysporum* f.sp. *cubense,* on the above ground dry mass of banana plants without the addition of lime and with different NO_3^-:NH_4^+ ratios. Values followed by the same letter do not differ significantly ($P<0.05$). SEM = 0.43, LSD = 1.21, CV = 42.5%.

Different results were obtained in the treatments that received lime, although these observations were not consistent throughout the various nitrogen treatments (Figure 3.4). With the addition of lime, an increase in nitrogen application at a consistent NO_3^-:NH_4^+ ratio of 14:1 resulted in a significant increase in plant dry mass of 86% from the lowest to the highest nitrogen level. A highly significant increase of 174% in plant growth was also obtained with an increase in NO_3^-:NH_4^+ ratio from 9:6 to 14:1 when applied at the highest nitrogen level. The lowest nitrogen application resulted in a non-significant yet clear increase of 76% in plant dry mass from the lowest (9:6) to the second (11:4) NO_3^-:NH_4^+ ratio. The intermediate

nitrogen application did not result in any distinct differences in plant dry mass, regardless of the NO_3^-:NH_4^+ ratio that was applied.

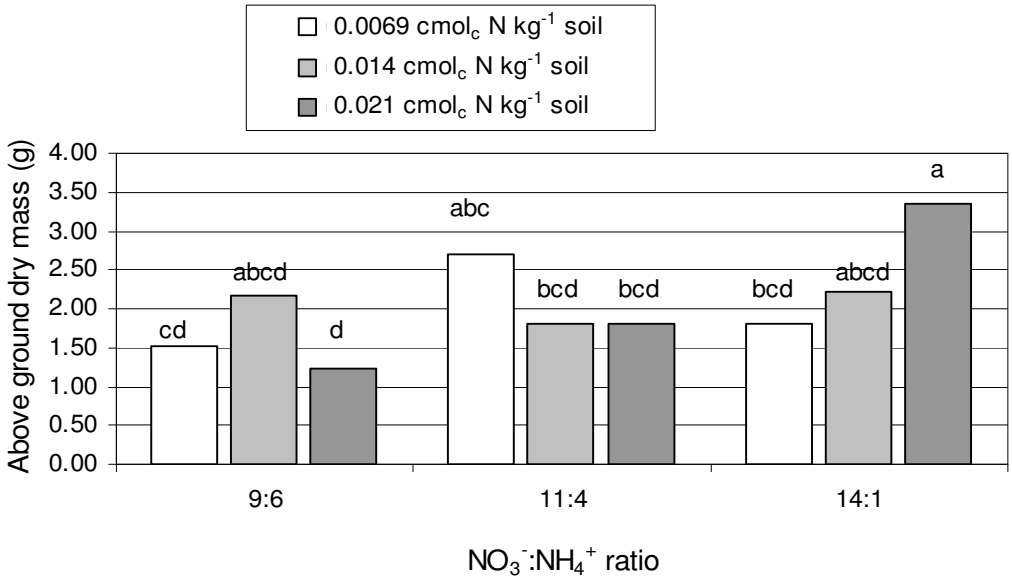

FIGURE 3.4. The effect of three N-levels applied every 3 weeks to pots with soil inoculated with *Fusarium oxysporum* f.sp. *cubense,* on the above ground dry mass of banana plants with the addition of lime and with different NO_3^-:NH_4^+ ratios. Values followed by the same letter do not differ significantly ($P<0.05$). SEM = 0.43, LSD = 1.21, CV = 42.5%.

To achieve the best plant growth in the presence of *Foc* and Fusarium wilt in an unlimed soil, low nitrogen should be applied at a NO_3^-:NH_4^+ ratio of 9:6. The opposite is true when the soil is limed, in which case high nitrogen levels mostly in the form of NO_3^- generally resulted in higher plant growth. This corresponds to the finding of Woltz & Jones (1981) that low NH_4^+-N and high NO_3^--N application was more favourable to plant growth by reducing the development of Fusarium wilt. It was expected that an increase in the amount of NO_3^--N applied would positively influence the growth of the banana plants when considering the work of Walker (1946) and Woltz & Jones (1973). In both instances, these researchers found that an increase in NO_3^--N decreased or suppressed Fusarium wilt in cotton, pea and tomato.

The addition of lime and NO_3^--N was suggested to raise the soil pH and decrease the virulence of the pathogen (Engelhard, 1989). It could also lower the concentration of nutrients such as phosphorous, magnesium, sulphur and possibly copper in the soil. However, in this study, the

addition of lime did not significantly raise the pH of the soil, which suggests that the Ca itself played a role in the observed results. Peng *et al.* (1999) found that the addition of $CaCO_3$ reduced chlamydospore germination and that smaller amounts of $CaCO_3$ were the most effective. Although it has also been stated that Ca nutrition can have a profound effect on vascular colonisation by the pathogen, it is not yet clear how Ca works (Beckman, 1987). It has been suggested that Ca has an effect on the composition of cell walls of the host plant, which makes them more resistant to penetration by facultative pathogens (Beckman, 1987). The effect of Ca on plant growth in the presence of Fusarium wilt, however, needs to be investigated further and the effects of Ca and pH need to be separated.

The Effect of Lime and Nitrogen Application on N-uptake

According to the factorial ANOVA (Appendix E), the interaction between pH and nitrogen level accounted for the highest percentage variance in the %N level in plants, with a SS% of 18.04%, followed by the nitrogen level main effect (SS% = 11.12%) and the nitrogen level x nitrogen source interaction (SS% = 10.13%).

Liming of the soil resulted in significant differences in nitrogen uptake by the plants at the lowest nitrogen application (Table 3.8; Figure 3.5). At this nitrogen level, the amount of plant nitrogen in the unlimed treatments was higher (15%, 10% and 7% for NO_3^-:NH_4^+ ratios of 9:6, 11:4 and 14:1 respectively) than in the limed treatments. This difference, therefore, decreased with an increase in the NO_3^-:NH_4^+ ratio. In the higher nitrogen applications, liming did not significantly affect the %N in the plants, except at the highest N-application with the highest NO_3^-:NH_4^+ ratio of 14:1. In this treatment, liming resulted in an 11% higher %N in plants, relative to that of the unlimed soil.

In the unlimed treatments, the amount of nitrogen in the plants in the lowest N-application treatments showed no difference, regardless of the NO_3^-:NH_4^+ ratio that was applied (Figure 3.6). The amount of nitrogen in these treatments was also significantly higher than that of the higher N-applications. An increase from the lowest to the highest nitrogen application resulted in a decrease in plant nitrogen of 9, 6 and 18%, respectively, for the NO_3^-:NH_4^+ ratios of 9:6, 11:4 and 14:1. A change in the applied NO_3^-:NH_4^+ ratio only affected the %N in the plants at the highest nitrogen application where a ratio increase from 9:6 to 14:1 resulted in a significant 10% decrease in nitrogen content in the plants.

TABLE 3.8. Average %N in the above ground banana plant material grown in pots with soil treated with different fertiliser combinations and inoculated with *Fusarium oxysporum* f.sp. *cubense*. Values followed by the same letter do not differ significantly (P<0.05). SEM = 0.09, LSD = 0.26, CV = 6.1%.

Nitrogen application		%N	
Amount	NO_3^-:NH_4^+ ratio	Without Lime	Lime
7.7 mg N	9:6	3.32^{abc}	2.81^{fg}
7.7 mg N	11:4	3.35^{ab}	3.03^{def}
7.7 mg N	14:1	3.36^{a}	3.13^{abcde}
15.4 mg N	9:6	3.08^{cde}	3.09^{bcde}
15.4 mg N	11:4	2.87^{efg}	2.97^{defg}
15.4 mg N	14:1	2.99^{defg}	2.94^{defg}
23.1 mg N	9:6	3.03^{def}	3.03^{def}
23.1 mg N	11:4	3.14^{abcd}	3.07^{cdef}
23.1 mg N	14:1	2.74^{g}	3.08^{cde}

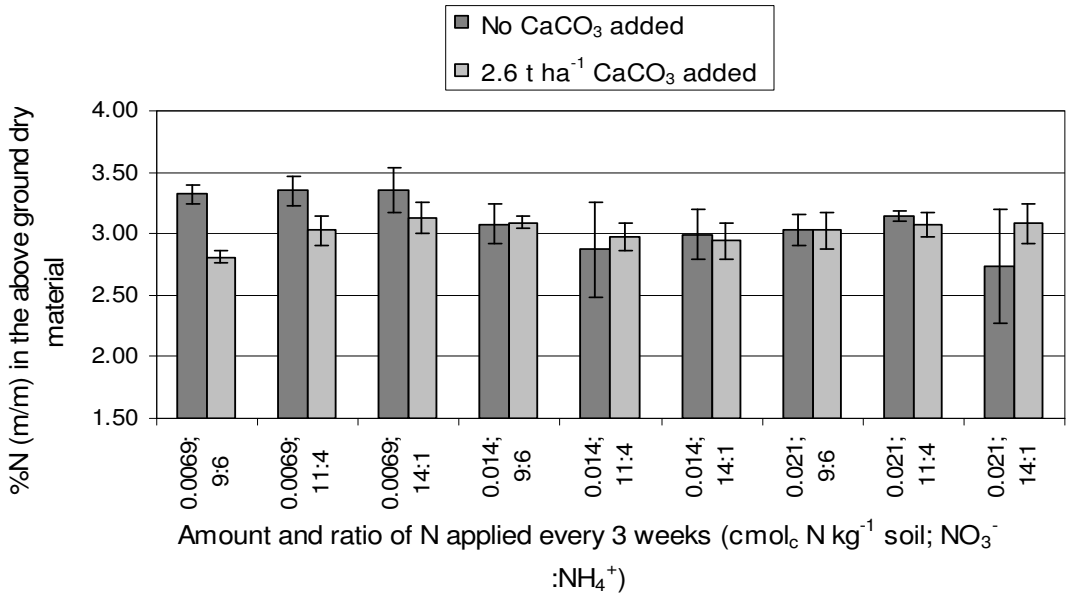

FIGURE 3.5. The average %N (m/m) in the above ground banana plant material grown in pots with soil, with and without the addition of lime, at different nitrogen applications, and inoculated with *Fusarium oxysporum* f.sp. *cubense*. The vertical bars indicate the standard deviations of the respective values. SEM = 0.09, LSD = 0.26, CV = 6.1%.

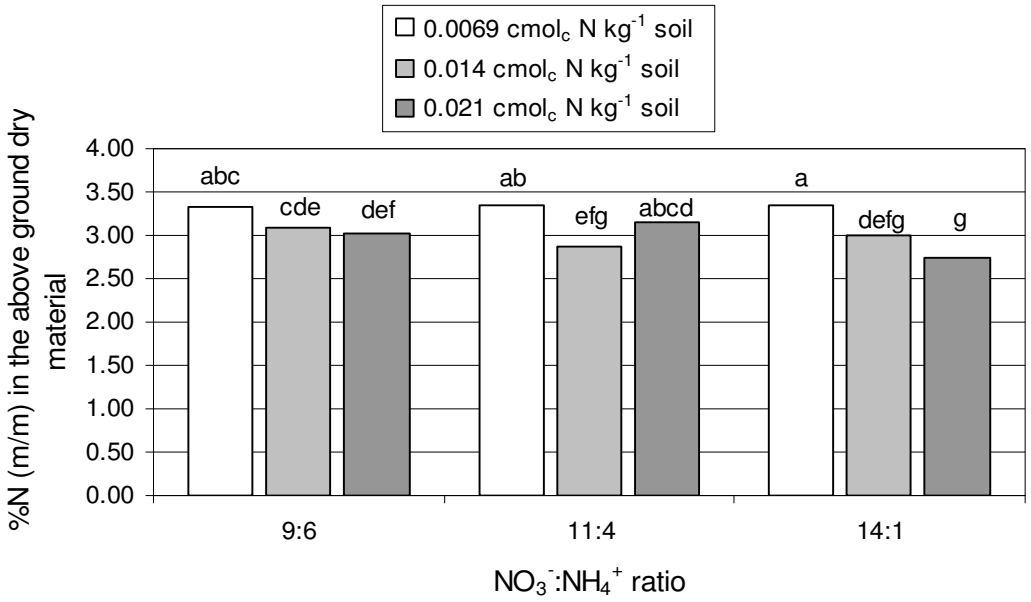

FIGURE 3.6. The effect of N-levels applied every 3 weeks to pots with soil inoculated with *Fusarium oxysporum* f.sp. *cubense,* on the %N (m/m) in the above ground banana plant material without the addition of lime and with different NO_3^-:NH_4^+ ratios. Values followed by the same letter do not differ significantly ($P<0.05$). SEM = 0.09, LSD = 0.26, CV = 6.1%.

With the addition of lime, the %N in the plants did not show obvious differences as a result of different nitrogen applications (Figure 3.7). The only significant difference was found at the lowest N-application with an 11% increase in the %N with an increase in the NO_3^-:NH_4^+ ratio from 9:6 to 11:4.

In the presence of the Fusarium wilt pathogen as well as the disease itself, low nitrogen application without the addition of lime resulted in significantly higher N-levels in the plants compared to when lime was added. On the other hand, the application of high nitrogen mostly in the form of NO_3^- resulted in the highest N-uptake when applied in conjunction with lime. This correlation between the growth and N-uptake results suggest that effective N-uptake is essential for better plant growth in the presence of Fusarium wilt, which in turn supports the view that nitrogen application can be used to improve plant growth in spite of the presence of *Foc*. It is also clear that high nitrogen application does not necessarily correlate with high nitrogen uptake by plants.

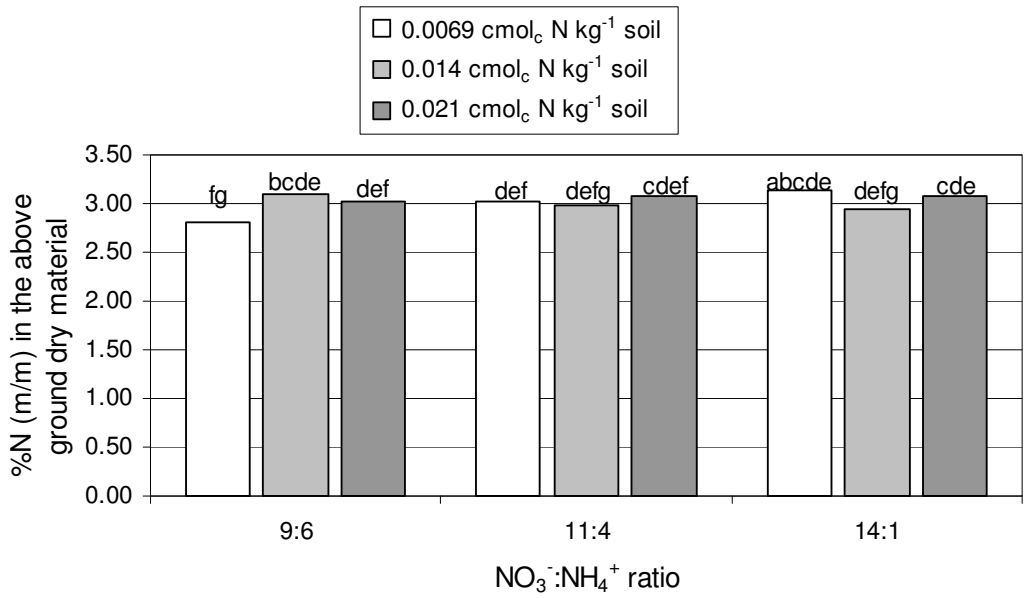

FIGURE 3.7. The effect of N-levels applied every 3 weeks to pots with soil inoculated with *Fusarium oxysporum* f.sp. *cubense,* on the %N (m/m) in the above ground banana plant material with the addition of lime and with different NO_3^-:NH_4^+ ratios. Values followed by the same letter do not differ significantly (P<0.05). SEM = 0.09, LSD = 0.26, CV = 6.1%.

HYDROPONIC TRIAL

The control treatments used in this trial were only conducted at one nitrogen level and NO_3^- :NH_4^+ ratio equivalent to that of the normal Hoagland 2 nutrient solution. It was therefore not conducted in the factorial design used in the rest of the treatments. For this reason, the control treatments could not be included in the factorial design used for statistical analysis. The controls are nonetheless included in the results below as an illustration of the differences in plant growth and nitrogen uptake between infected and uninfected plants.

Internal Disease Symptoms

Internal symptoms of Fusarium wilt developed in all the plants in the containers inoculated with *Foc*, while, as expected, no internal disease symptoms developed in the control treatments. The treatment of banana plants with different applications of nitrogen and varying levels of pH, therefore, did not affect the infection status of the plants with *Foc*. Also, a high

disease incidence did not correlate with a low above ground dry matter of the plants (Figure 3.8).

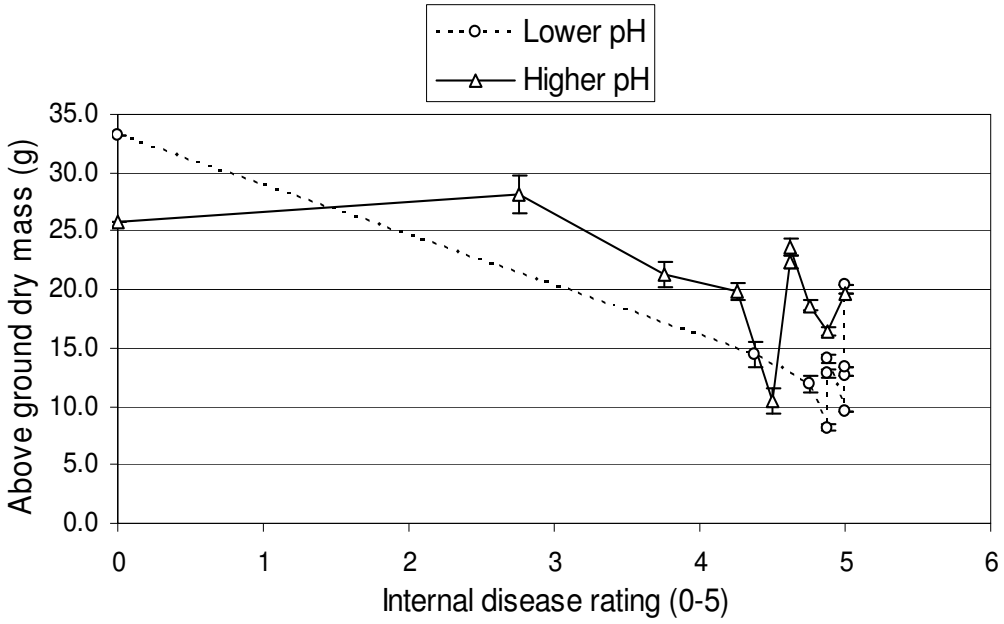

FIGURE 3.8. The internal disease rating of banana plants grown in containers with a water solution treated with three different nitrogen-levels and NO_3^-:NH_4^+ ratios, and inoculated with *Fusarium oxysporum* f.sp. *cubense,* in relation to their above ground dry mass. The internal disease symptoms were rated according to the degree of discolouration of the rhizome just above the root entry level, with a rating of 0 for no discolouration, up to a rating of 5, indicating that the total inner rhizome was discoloured. The vertical bars indicate the standard deviations of the respective values.

Effect of Manipulation of $KHCO_3$ on Solution pH

At the time of inoculation (after 1 week of growth in the replaced nutrient solution), the pH levels had dropped from 6 in the low pH treatments and 7 in the high pH treatments, to an average of 4.9 and 5.5, respectively. The high pH treatments, however, maintained a slightly higher pH than the low pH treatments.

Effect of pH and Nitrogen Treatments on Above Ground Dry Matter Production

According to the factorial ANOVA (Appendix F) the main effects of both pH (SS% = 26.27%) and nitrogen level (SS% = 16.95%) were highly significant.

Despite the high infection rate, therefore, significant yield differences were observed between the different treatments (Table 3.9), which could be attributed to two possible factors. The first factor being that better plant growth was still achieved due to certain treatments as discussed below, despite the presence of the pathogen in the plants. The second factor was a noticeable difference in the growth of the plants already established during the four weeks of growth prior to inoculation of the nutrient solutions with *Foc*. The different pH and nitrogen treatments were applied from the beginning of the trial and therefore already had an effect on plant growth prior to inoculation. Although it could be argued that a standard nutrient solution should be applied to all the pots prior to inoculation, the difference in plant growth due to the different treatments indicated that certain nutrient conditions gave plants a head start in order to cope better with later infection with *Foc*.

TABLE 3.9. Average dry mass of the above ground banana plant material grown in containers with a water solution treated with different pH and nitrogen combinations and inoculated with *Fusarium oxysporum* f.sp. *cubense*. Values followed by the same letter do not differ significantly ($P<0.05$). SEM = 2.45, LSD = 7.09, CV% = 30.3%.

Nitrogen application		Dry mass (g)	
Amount ($mmol_c\,N\,L^{-1}$)	NO_3^-:NH_4^+ ratio	Low pH	High pH
7.5 $mmol_c\,N\,L^{-1}$	9:6	13.29^{efghi}	21.26^{abcd}
7.5 $mmol_c\,N\,L^{-1}$	11:4	14.04^{efghi}	19.80^{bcdef}
7.5 $mmol_c\,N\,L^{-1}$	14:1	14.40^{defghi}	28.10^{a}
15 $mmol_c\,N\,L^{-1}$	9:6	20.32^{bcde}	19.71^{bcdef}
15 $mmol_c\,N\,L^{-1}$	11:4	12.57^{ghi}	23.57^{ab}
15 $mmol_c\,N\,L^{-1}$	14:1	12.76^{fghi}	22.32^{abc}
22.5 $mmol_c\,N\,L^{-1}$	9:6	11.95^{ghi}	16.46^{cdefgh}
22.5 $mmol_c\,N\,L^{-1}$	11:4	8.21^{i}	18.65^{bcdefg}
22.5 $mmol_c\,N\,L^{-1}$	14:1	9.54^{hi}	10.39^{hi}

As expected, the uninfected control treatments generally yielded a higher dry mass than the infected plants (Figure 3.9). In the inoculated treatments, the low pH treatments generally yielded a lower dry mass relative to the high pH treatments. At the lowest nitrogen application, the plant dry mass was significantly lower (37% and 49%) in the low pH treatments, relative to the high pH treatments at NO_3^-:NH_4^+ ratios of 9:6 and 14:1,

respectively. The magnitude of these differences in plant dry mass therefore increased with an increase in the NO_3^-:NH_4^+ ratio applied. At the intermediate nitrogen application level an increase in the NO_3^-:NH_4^+ ratio from 9:6 to 14:1 also resulted in an increase in the difference in dry mass between the low pH and high pH treatments. At a ratio of 9:6, the low pH treatment resulted in a slightly higher dry mass than the high pH treatment, while at a ratio of 14:1 the dry mass of the high pH treatment was higher than that of the low pH treatment by a significant 43%. The only significant difference at the highest nitrogen application was found at a NO_3^-:NH_4^+ ratio of 11:4, with a significant 56% higher dry mass in the high pH treatment, relative to the low pH treatment.

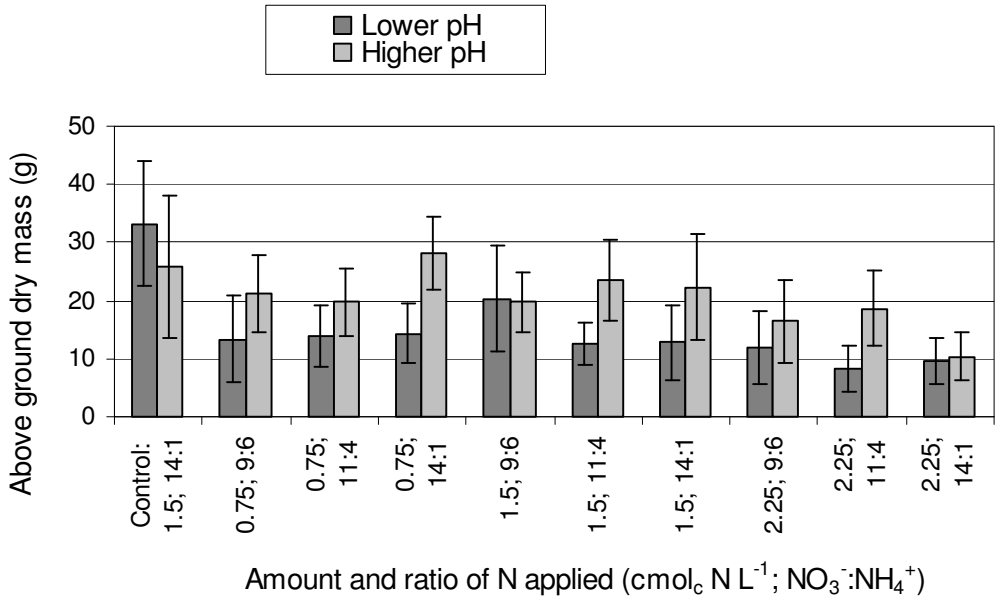

FIGURE 3.9. Average above ground mass of banana plants grown in containers with a water solution inoculated with *Fusarium oxysporum* f.sp. *cubense*, and treated with low pH and high pH treatments and different nitrogen applications. The vertical bars indicate the standard deviations of the respective values. SEM = 2.45, LSD = 7.09, CV% = 30.3%.

A slightly higher pH in the nutrient solution was more beneficial to plant growth in the presence of *Foc*, which confirms that the manipulation of growth medium pH can be successful in altering the growth of banana plants despite the presence of *Foc* in the plants. Especially in the case of using soil as the growth medium, it was not clear whether liming influenced plant growth due to a change in pH or due to the addition of Ca in the form of lime. However, in the case of the hydroponic trial, no extra Ca was added in order to increase the

pH of the solution. The effect of added Ca was therefore eliminated. The effect of the higher pH is hence suggested to be due to the direct negative effect of lower pH on the pathogen by creating a less suitable growth environment. These results warrant investigations into the effect of changes in soil pH on infection and growth of banana plants in the field.

The dry mass of the control plants in the low pH treatments was markedly higher than that of all the other treatments with a value of 33.16 g (Figure 3.10). This indicates that, although the pH of the nutrients solutions was lower than the intended level at the time of inoculation with *Foc*, banana plants were still able to achieve good growth in the absence of the Fusarium wilt pathogen. Differences in the dry mass of plants between the different nitrogen levels were not significant. In terms of the nitrogen source, however, at a nitrogen application of 15 mmol N L^{-1}, an increase in the NO_3^-:NH_4^+ ratio from 9:6 to 14:1 resulted in a significant decrease in dry matter production of 37%.

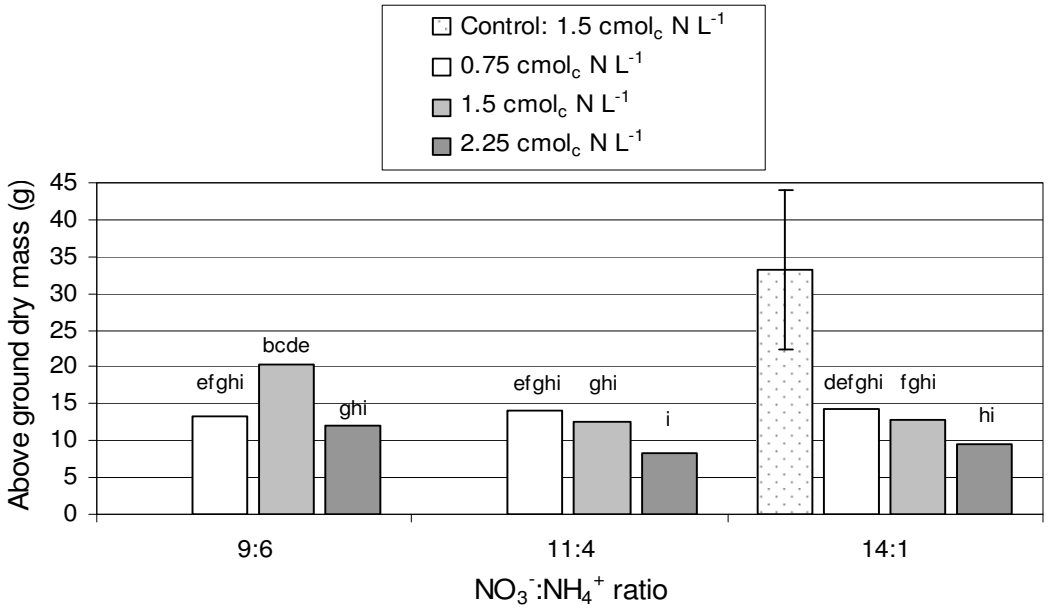

FIGURE 3.10. The effect of different N-levels on the above ground mass of banana plants grown in containers with a water solution inoculated with *Fusarium oxysporum* f.sp. *cubense,* at low pH and with different NO_3^-:NH_4^+ ratios. Values followed by the same letter do not differ significantly (P<0.05). The vertical bar indicates the standard deviation of the control. SEM = 2.45, LSD = 7.09, CV% = 30.3%.

In the high pH treatments the application of 7.5 mmol N L^{-1} at a NO_3^-:NH_4^+ ratio of 14:1 resulted in a slightly higher dry mass than the control treatment (Figure 3.11). This

exceptional growth of plants in spite of low nitrogen application and infection with *Foc* indicates that there is merit in the investigation of lower nitrogen application in order to contribute to the management Fusarium wilt of banana. An increase in the NO_3^-:NH_4^+ ratio from 9:6 to 14:1 applied at a nitrogen level of 7.5 mmol N L^{-1} resulted in a 32% increase in dry matter production. However, at a nitrogen level of 22.5 mmol N L^{-1}, an increase in the NO_3^-:NH_4^+ ratio from 9:6 to 14:1 resulted in a 37% decrease in dry matter production. An increase in the amount of nitrogen applied from 7.5 to 22.5 mmol N L^{-1} resulted in a highly significant decrease in dry matter production of 63% at a NO_3^-:NH_4^+ ratio of 14:1.

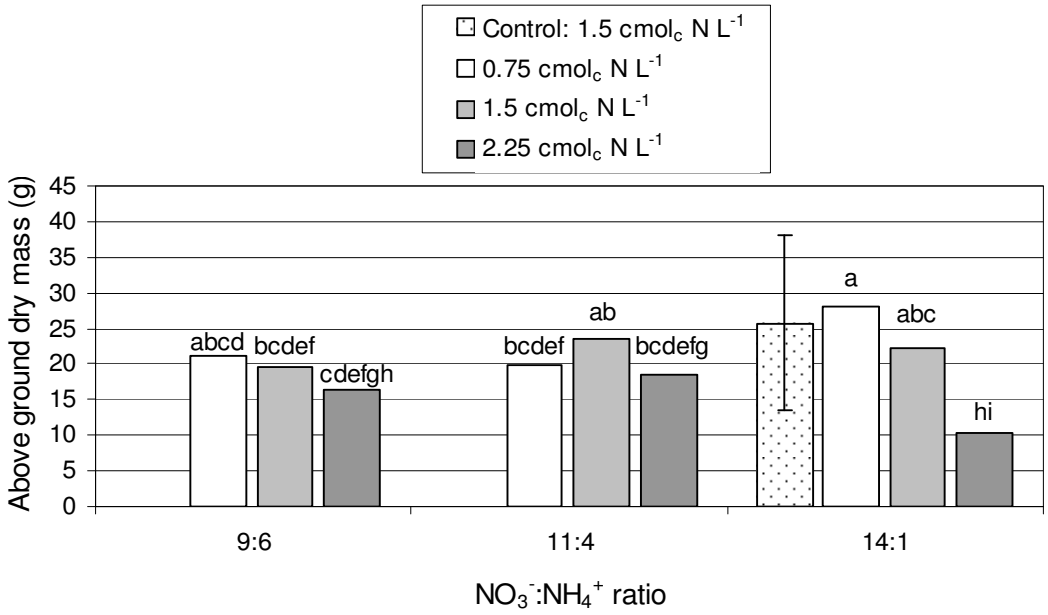

FIGURE 3.11. The effect of different N-levels applied to a water solution in containers inoculated with *Fusarium oxysporum* f.sp. *cubense,* on the above ground mass of banana plants at high pH and with different NO_3^-:NH_4^+ ratios. Values followed by the same letter do not differ significantly ($P<0.05$). The vertical bar indicates the standard deviation of the control. SEM = 2.45, LSD = 7.09, CV% = 30.3%.

In both the lower and higher pH treatments, an increase in the amount of nitrogen applied generally resulted in a decrease in the dry matter production of the banana plants and the lowest nitrogen application therefore resulted in the best plant growth. These results support the hypothesis that over-fertilisation with nitrogen may be a contributing factor in the development of Fusarium wilt of banana.

It is evident that less significant results were obtained in this trial compared to the soil pot trial and that the absence of a rhizosphere generally evened out the results across the different nitrogen treatments, both at the lower and the higher pH levels. Statistically, the effect of nitrogen source on the observed results were also not significant, either as a main effect or as part of an interaction. In the soil pot trial, however, the interaction of nitrogen source with pH and nitrogen level, respectively, were statistically found to be important in dry matter production. It is therefore suggested that the presence of the rhizosphere in the soil plays an important role in the observed effect that liming and nitrogen application has on Fusarium wilt, as suggested by Engelhard (1989).

Effect of pH and Nitrogen Treatments on N-content of Above Ground Material

Statistically, highly significant results were found in the %N in the plants (Appendix G). The main effect of nitrogen level was showed to account for the highest percentage of variance in the observed results with a SS% of 37.23%, while the interaction of nitrogen level and pH accounted for the second highest percentage of variance with a SS% of 20.83%. The three-way interaction between nitrogen level, pH and nitrogen source was also significant.

The %N in the dry plant material was consistently higher in the low pH treatments compared to the high pH treatments for the 7.5 and 15 mmol N L^{-1} treatments (Table 3.10; Figure 3.12). At the lowest nitrogen application of 7.5 mmol N L^{-1}, this difference increased from an insignificant 5% at a NO_3^-:NH_4^+ ratio of 9:6, to significant differences of 13% and 22%, respectively, at ratios of 11:4 and 14:1. With a nitrogen application of 15 mmol N L^{-1}, the %N in the low pH treatments were also significantly higher by 24% and 14%, respectively, for the NO_3^-:NH_4^+ ratios of 11:4 and 14:1, relative to the high pH treatments. The opposite was true of the 22.5 mmol N L^{-1} treatments, where the %N in the low pH treatments was consistently lower than in the high pH treatments. Once again this difference increased from a non-significant 1% at a NO_3^-:NH_4^+ ratio of 9:6, to a significant 15 and 18% at ratios of 11:4 and 14:1, respectively.

TABLE 3.10. Average %N in the above ground banana plant material grown in containers with a water solution treated with different fertiliser combinations, and inoculated with *Fusarium oxysporum* f.sp. *cubense*. Values followed by the same letter do not differ significantly (P<0.05). SEM = 0.14, LSD = 0.39, CV% = 8.2%.

Nitrogen application		%N	
Amount ($mmol_c\ N\ L^{-1}$)	NO_3^-:NH_4^+ ratio	Low pH	High pH
7.5 $mmol_c\ N\ L^{-1}$	9:6	3.31^{cde}	3.16^{de}
7.5 $mmol_c\ N\ L^{-1}$	11:4	3.50^{bcd}	3.04^{ef}
7.5 $mmol_c\ N\ L^{-1}$	14:1	3.26^{cde}	2.55^{g}
15 $mmol_c\ N\ L^{-1}$	9:6	3.34^{cde}	3.05^{ef}
15 $mmol_c\ N\ L^{-1}$	11:4	3.53^{bcd}	2.68^{fg}
15 $mmol_c\ N\ L^{-1}$	14:1	3.19^{cde}	2.75^{fg}
22.5 $mmol_c\ N\ L^{-1}$	9:6	3.81^{ab}	3.84^{ab}
22.5 $mmol_c\ N\ L^{-1}$	11:4	3.56^{bc}	4.18^{a}
22.5 $mmol_c\ N\ L^{-1}$	14:1	3.18^{cde}	3.88^{ab}

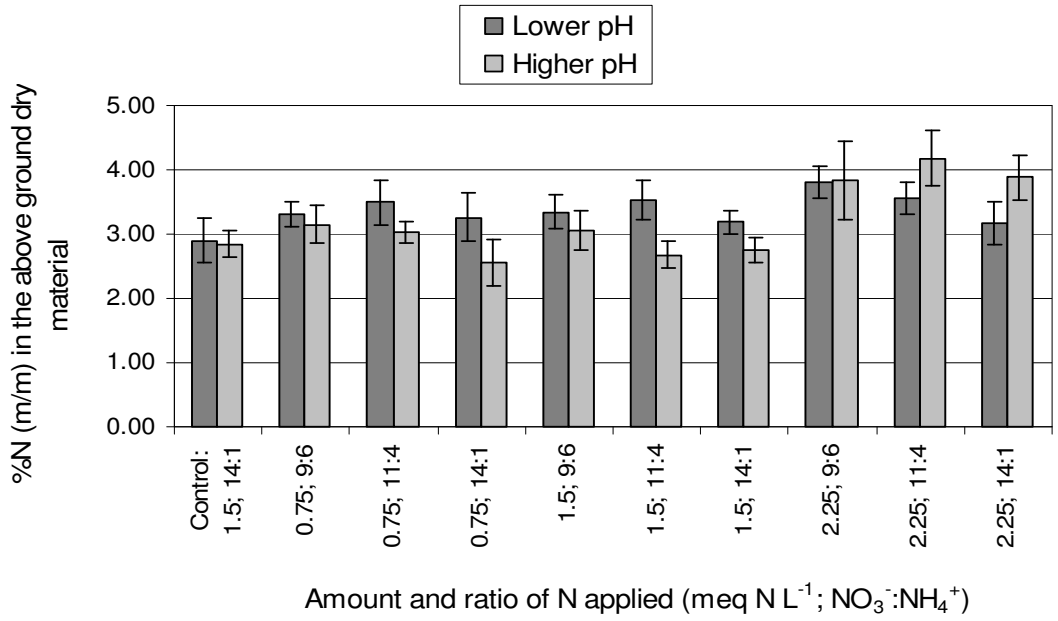

FIGURE 3.12. Average %N (m/m) in the above ground banana plant material grown in containers with a water solution inoculated with *Fusarium oxysporum* f.sp. *cubense,* at low pH and high pH levels and at different nitrogen applications. The vertical bars indicate the standard deviations of the respective values. SEM = 0.14, LSD = 0.39, CV% = 8.2%.

In the low pH treatments, the %N in the plant material was the lowest in the control treatments (Figure 3.13), which could be due to the dilution effect caused by the high dry matter production of the control plants. An increase in the NO_3^-:NH_4^+ ratio from 9:6 to 14:1 resulted in a significant decrease of 17% in the %N at an application of 22.5 mmol N L^{-1}, whilst at nitrogen applications of 7.5 and 15 mmol N L^{-1}, it had no influence on the %N in the plants. An increase in applied N from 7.5 to 22.5 mmol N L^{-1}, in turn, showed significant results with a NO_3^-:NH_4^+ ratio of 9:6, where the %N significantly increased by 13%, whereas at NO_3^-:NH_4^+ ratios of 11:4 and 14:1, an increase in applied N did not affect the %N in the plants.

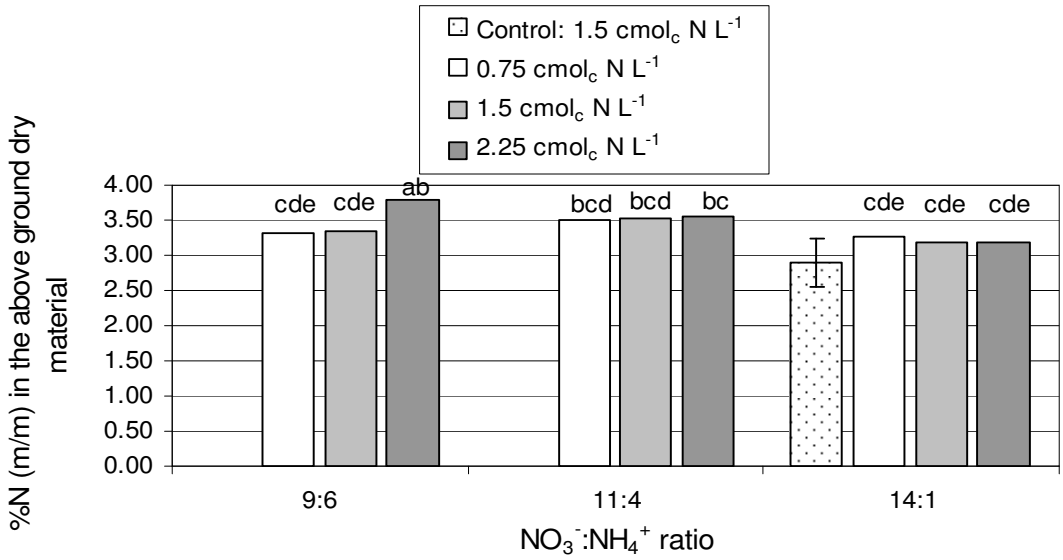

FIGURE 3.13. The effect of N-levels on the %N (m/m) in the above ground banana plant material grown in containers with a water solution inoculated with *Fusarium oxysporum* f.sp. *cubense,* at low pH and with different NO_3^-:NH_4^+ ratios. Values followed by the same letter do not differ significantly ($P<0.05$). The vertical bar indicates the standard deviation of the control. SEM = 0.14, LSD = 0.39, CV% = 8.2%.

This scenario changed in the high pH treatments (Figure 3.14). When the NO_3^-:NH_4^+ ratio was increased from 9:6 to 14:1, the %N in the plants decreased significantly by 19% at 7.5 mmol N L^{-1}. With an increase in the NO_3^-:NH_4^+ ratio from 9:6 to 11:4, the %N decreased by 12% at 15 mmol N L^{-1}. At 22.5 mmol N L^{-1}, an increase in the NO_3^-:NH_4^+ ratio did not influence the %N in the plants. However, at this pH level, the %N at 22.5 mmol N L^{-1} was significantly higher than all the other treatments, regardless of the NO_3^-:NH_4^+ ratio applied. An increase in

the amount of N applied from 7.5 to 22.5 mmol N L^{-1} resulted in a significant increase of 18%, 28% and 34% in the plants at NO_3^-:NH_4^+ ratios of 9:6, 11:4 and 14:1, respectively.

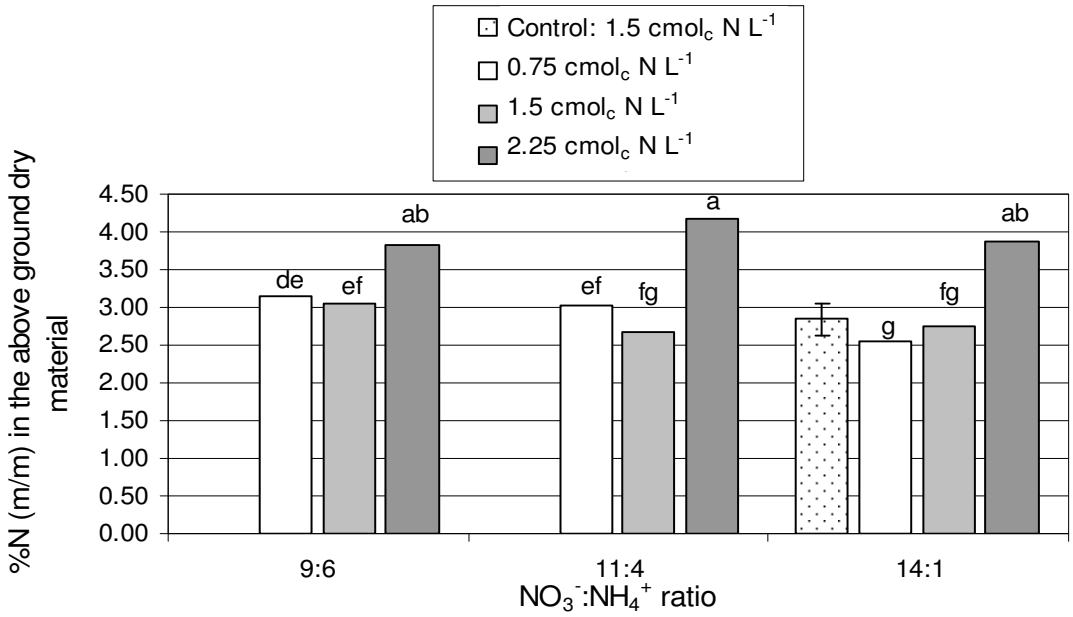

FIGURE 3.14. The effect of N-levels on the %N (m/m) in the above ground banana plant material grown in containers with a water solution inoculated with *Fusarium oxysporum* f.sp. *cubense,* at high pH and with different NO_3^-:NH_4^+ ratios. Values followed by the same letter do not differ significantly ($P<0.05$). The vertical bar indicates the standard deviation of the control. SEM = 0.14, LSD = 0.39, CV% = 8.2%.

The effect of pH on nitrogen uptake by the plants appears to be dependant on the amount of nitrogen applied. These results indicate that low to intermediate nitrogen application in conjunction with low pH resulted in the best nitrogen uptake. However, with high nitrogen application, a higher pH was essential for higher plant nitrogen levels. It is also apparent that the magnitude of the difference in nitrogen uptake by plants at different pH levels was dependent on the NO_3^-:NH_4^+ ratio at which such nitrogen was applied. The effect of pH on nitrogen uptake was the greatest when nitrogen was applied mostly in the form of NO_3^-.

In general, the low pH treatments did not show significant differences in nitrogen uptake due to different nitrogen applications. Only when high levels of nitrogen were applied did an increase in the NO_3^-:NH_4^+ ratio result in a decrease in nitrogen levels in the plants, indicating that high nitrogen applications should be applied as both NO_3^- and NH_3^+ to ensure good

nitrogen uptake. At a higher pH, on the other hand, the highest nitrogen application resulted in significantly higher nitrogen uptake than in the two lower applications. In this case, therefore, an increase in nitrogen application to the highest level correlated with a higher nitrogen uptake by plants.

CONCLUSION

Although the incidence of Fusarium wilt in Cavendish banana seedlings was not reduced by treatment of the soil with different N sources and different pH levels, plants reacted significantly differently to different treatments in terms of plant growth and nitrogen uptake. It was concluded from these results that, in order to achieve the best plant growth and nitrogen uptake in the presence of Fusarium wilt, the application of low N-levels containing both NO_3^- and NH_4^+ should not be accompanied by the application of lime. On the other hand, if lime is applied to the soil, nitrogen application should be at higher nitrogen levels and consist mostly of NO_3^--N.

The results from the hydroponic trial strongly indicate that the rhizosphere in the soil plays an important role in creating a micro-environment that affects the pathogen - an environment that was missing in this trial and resulted in less significant differences in plant growth due to the different treatments applied. Statistically, the effect of nitrogen source on plant growth in the hydroponic trial was also shown not to play an important role in the observed results, compared to that of the soil pot trial. A high application of nitrogen in the soil pot trial, mostly in the form of NO_3^-, may have increased the rhizosphere pH, which, together with the addition of lime, resulted in the best plant growth - an effect that was not observed in the hydroponic trial. The role of the rhizosphere can be evaluated and quantified in further studies in order to better understand the reasons behind the observed effects that nitrogen application and liming or pH-changes have on Fusarium wilt and the growth of plants in the presence of *Foc*.

Future field tests are essential to determine whether lower amounts of nitrogen could be added to the soil when soils are not limed. This may save on both liming and N-fertilisation costs. However, the effect of not liming the soil would also affect other soil parameters such as the availability of heavy metals and P, which were not determined in this trial. Field tests should also be done to determine whether the liming of soil does indeed require nitrogen to be applied in higher amounts and at higher NO_3^-:NH_4^+ ratios in order to be more beneficial to

plant growth. These results strongly indicate that effective liming and nitrogen management can improve the growth of banana plants infected with *Foc*. It is suggested that the application of nitrogen and manipulation of the soil pH level could be used in the development of an integrated program to manage Fusarium wilt of banana in South Africa.

CHAPTER 4

CHEMICAL ANALYSIS OF SOILS THAT ARE EITHER SUPPRESSIVE OR CONDUCIVE TO FUSARIUM WILT IN SOUTH AFRICA

INTRODUCTION

Areas exist in Kiepersol (Mpumalanga) that exhibit either suppressive or conducive properties towards Fusarium wilt of banana. These areas are characterised by high disease incidence, and either contain or border smaller areas where disease development is slower.

Suppressive soils are defined as soils in which disease severity is reduced, despite the presence of a susceptible host, a virulent pathogen in sufficient concentration, and favourable environmental conditions (Louvet *et al*., 1981; Peng *et al*., 1999). Conducive soils, on the other hand, are soils with no ability to reduce disease severity (Peng *et al*., 1999). Suppressive and conducive soils to Fusarium wilt of banana, caused by *Fusarium oxysporum* f.sp. *cubense* (*Foc*), were first described in Central America in the 1930's (Stover, 1962). Since then, very little research has been done on soil suppressiveness to Fusarium wilt of banana. Properties of soils suppressive and conducive to various other Fusarium wilt diseases, however, were well investigated, and physical, chemical and biological differences between these soils were determined (Scher & Baker, 1982; Duskova & Prokinova, 1989; Höper *et al.;* 1995).

Previous investigations on the chemical composition of suppressive and conducive soils indicated that, in suppressive soils, the cation exchange capacity (Domínguez *et al.*, 1996), soil solution pH (Domínguez *et al*., 2001) and soluble Na (Domínguez *et al.*, 1996; 2001) was higher than in conducive soils. The use of the soil Na indices such as exchangeable sodium percentage (ESP), soluble sodium (SS) and sodium adsorption ratio (SAR) to predict soil conduciveness or suppressiveness to *Foc* was investigated by Domínguez *et al.* (2003). Their results indicated that the SS and SAR was always greater in suppressive than in conducive soils, and thus the SS and SAR can be satisfactory indices to study the influence of Na concentrations on the incidence of Fusarium wilt.

Analysis of field soils showed that diseased areas contained a higher amount of exchangeable NH_4^+ than non-diseased areas (Domínguez *et al.*, 1996). In a study in Carnarvon, Western

Australia, Peng *et al.* (1999) found that soils suppressive to Fusarium wilt of banana contained a higher concentration of calcium, magnesium, sodium and potassium, and had a higher electrical conductivity (EC) compared to the conducive soils. They also found that a decrease in the amount of available Fe in these soils resulted in a decrease in disease severity in both the suppressive and conducive soils. Scher & Baker (1982) and Höper *et al.* (1995) found similar effects due to differences in Fe-availability in terms of Fusarium wilt of flax. These results were ascribed to competition between the pathogen and the plants for the available Fe, as different Fe sources can either reduce or increase Fe-availability, depending on their degree of chelation. The addition of a combination of Mn and Zn lignosulfonates and a combination of Fe and Zn lignosulfonates to a limed soil, reversed the disease suppressing effect of liming on disease incidence in tomato. As the rates of application of these micro nutrients were increased, the incidence and severity of wilt also increased (Jones & Woltz, 1970).

The addition of $CaCO_3$ to soil increased the degree of soil suppressiveness to Fusarium wilt of flax (Höper *et al.*, 1995), and significantly reduced chlamydospore germination in soils both suppressive and conducive to Fusarium wilt of banana (Peng *et al.,* 1999). The addition of both $Ca(OH)_2$ and $CaSO_4$ were also successful in reducing the degree of chlamydospore germination of the banana wilt pathogen (Peng *et al.,* 1999).

A soil pH of near or slightly above 7 was generally found to be the least optimal for Fusarium wilt development. Fusarium wilt is generally a disease associated with more acidic, sandy soils, rather than heavier soils with higher pH levels (Woltz & Jones, 1981). The pH of soil in a field suppressive to Fusarium wilt of banana was higher than in the conducive soil (Peng *et al.,* 1999), while an increase in soil pH resulted in a reduced number of carnations infected with Fusarium wilt (Duskova & Prokinova, 1989).

It is important to note that soil suppressiveness is not attributed to any single soil factor, but is a combination and interaction of all soil factors, namely soil microbiological, -chemical and –physical characteristics. Understanding the dynamics of suppressive soils and the interaction of the respective soil characteristics, however complex, can therefore contribute substantially to the eventual manipulation of soils in order to suppress or reduce Fusarium wilt of banana.

The objective of this study was to analyse the chemical properties of soils in Kiepersol, South Africa, with apparent suppressiveness to Fusarium wilt of banana. In the soils under consideration, disease development was much slower than in other soils of the same fields.

MATERIALS AND METHODS

SELECTION OF SOILS SUPPRESSIVE TO FUSARIUM WILT OF BANANA

Three areas were identified in Kiepersol (Mpumalanga) that show signs of suppressiveness to Fusarium wilt of banana. The first field (Field 1) has been infected with Fusarium wilt for approximately 15 years, but contains a rectangular area that is not affected by the disease, despite the regular movement of people, field equipment and water. In the second field (Field 2), rhizomes of Cavendish bananas from a heavily diseased field were planted to an area of about 0.5 ha that had been heavily fertilised with chicken manure before and after planting. Despite being surrounded by fields severely affected by Fusarium wilt, this site has not developed Fusarium wilt since planting, and therefore seems to be suppressing the disease. In the third field (Field 3), an area of approximately 100 m^2 remained unaffected by Fusarium wilt, even though dying plants have surrounded it for at least 5 years. Machinery and soil is regularly moved through this area, suggesting that contamination of this soil must have taken place.

SOIL SAMPLING

In each field, 10 points were sampled in the suppressive and the adjacent conducive area, respectively. Nine of these points were sampled with an auger, while a profile pit was dug at the tenth sampling point. Each profile pit was dug in the most accessible spot, since a back actor was used for the digging.

An imaginary boundary was identified between each suppressive and conducive area, and sampling started 3 m on either side of this boundary. Auger samples were taken 3 m apart on a grid that fit into the area. In Field 1, due to the rectangular shape of the suppressive area, eight auger samples were taken in a 9 x 3 m grid, with the ninth sample taken in the middle of the grid. The same grid was used in the conducive area. In Fields 2 and 3, nine auger samples were taken in a 6 x 6 m grid in the suppressive and conducive areas.

A sample of 0–300 mm deep was taken at each point (10 samples), since approximately 85% of the vertical root volume occurs in the top 300 mm of soil (Robinson, 1996). However, some banana roots also occur deeper in the soil and five samples were taken at 300-600 mm and 600-900 mm, respectively, to shed further light on the nutrient status of the soil. Four points on each grid were randomly selected for the deeper samples, and the fifth sample was taken from the profile pit.

SOIL ANALYSIS

All collected soils samples were chemically analysed for NO_3^-- and NH_4^+-N, phosphate (P), potassium (K), magnesium (Mg), calcium (Ca), sodium (Na), iron (Fe), copper (Cu), manganese (Mn) and zinc (Zn). The pH(H_2O) and pH(KCl) of the soil samples were also determined.

Determination of N-content

The determination of both NO_3^- and NH_4^+ content in the soil was done through steam distillation (Non-Affiliated Soil Analysis Work Committee, 1990). The soil samples were oven-dried overnight at 40ºC and 100 ml of a 1 N solution of KCl was added to 50 g of the soil. The suspension was mechanically shaken for 1 hour, filtered and the N-content of the filtrate was determined.

For the determination of NH_4^+, 15 ml of a 50% (v/v) NaOH solution was added to 50 ml of the filtrate obtained above and distilled for 7 minutes into 25 ml boric acid. The distillate was titrated with 0.01 M HCl and the amount of NH_4^+ was calculated. For the determination of NO_3^-, a spatula tip of Davarda Alloy was added to the distilled sample and left to stand until the solution was fully reduced. The solution was then distilled for 7 minutes into 25 ml boric acid and the distillate was titrated with 0.01 M HCl. The amount of NO_3^- was then calculated.

Determination of Phosphate

The phosphate determination was done according to the Bray-1 method (Non-Affiliated Soil Analysis Work Committee, 1990). Fifty ml of Bray-1 solution was added to 6.67 g of soil and was shaken manually for 60 seconds. Two drops of flocculant was added and the solution was

filtered immediately through Whatman no 2V filter paper. The amount of phosphate in the filtrate was determined with an Auto Analyzer.

Determination of Ca, Mg, K and Na

The macro nutrients were extracted from the soil with a 1 M ammonium acetate solution at pH 7. The extraction was made from 5 g of soil and 50 ml of the 1 M NH_4OAc solution at pH 7, which was shaken at 180 oscillations per minute for 30 minutes. The solution was filtered and the cations in the filtrate were determined by flame emission spectroscopy (Non-Affiliated Soil Analysis Work Committee, 1990).

Determination of Cu, Fe, Mn and Zn,

The micro nutrient concentration in the soil was determined by adding 15 ml of a 0.02 M di-ammonium EDTA solution to 5 g of soil. The solution was shaken at 180 oscillations per minute for 60 minutes. The solution was filtered, whereafter the Ca and Mg in the filtrate was determined by atomic absorbtion spectorphotometry and the K and Na by flame emission spectroscopy (Non-Affiliated Soil Analysis Work Committee, 1990).

Determination of pH

The pH(H_2O) and pH(KCl) were determined using a 1:2.5 extract. Twenty g of soil and 50ml of de-ionised water or 1M KCl was stirred rapidly for 5 seconds with a glass rod. The mixture was stirred again after 30 minutes, after which the pH was determined with a calibrated pH meter with the electrodes positioned in the supernatant (Non-Affiliated Soil Analysis Work Committee, 1990).

STATISTICAL ANALYSIS

Student's two-sample unpaired t-test was used to test for differences between the suppressive and conducive soils per area and soil depth. Significance was obtained at the 5 % level of significance ($P < 0.05$) (Snedecor & Cochran, 1980).

RESULTS AND DISCUSSION

DETERMINATION OF MACRO ELEMENTS AND PHOSPHATE

The levels of macro nutrients differed between fields and between sampling depths (Appendix H), with significant differences occurring in some of the results (Appendix I). In the 0-30mm soil layer, Fields 1 and 3 either showed no difference or the results were higher in the conducive soil compared to the suppressive soil, although these differences were not significant (Table 4.1). These results are therefore contrary to those reported by previous researchers (Domínguez *et al.*, 1996, 2001; Peng *et al.,* 1999) who found higher levels of Ca (Figure 4.1), K (Figure 4.2), Mg (Figure 4.3) and Na (Figure 4.4) in suppressive soils than in conducive soils. Field 2, however, supported the results of the above-mentioned researchers with significantly higher levels of Ca, K, Mg and Na in the suppressive soil compared to the conducive soil. It is possible that the application of chicken manure in the suppressive area of Field 2 had contributed to the different results found in this field compared to Fields 1 and 3.

TABLE 4.1. The nutrient statuses of the top 300 mm of Fusarium wilt suppressive and conducive soils in three banana fields. Each element or property is indicated as higher (H) or lower (L) in the suppressive compared to the conducive soil of that field. No difference is indicated by "-" in both the suppressive and the conducive column of the appropriate field.

Property	Unit	Field 1		Field 2		Field 3	
		Suppr†	Cond‡	Suppr†	Cond‡	Suppr†	Cond‡
Ca	$cmol_c\ kg^{-1}$	-	-	H	L	L	H
K	$cmol_c\ kg^{-1}$	L	H	H	L	L	H
Mg	$cmol_c\ kg^{-1}$	-	-	H	L	L	H
Na	$cmol_c\ kg^{-1}$	L	H	H	L	L	H
P	$mg\ kg^{-1}$ soil	L	H	H	L	H	L
NO_3^-	$cmol_c\ kg^{-1}$	L	H	H	L	H	L
NH_4^+	$cmol_c\ kg^{-1}$	L	H	H	L	H	L
$pH(H_2O)$		L	H	H	L	L	H
Cu	$mg\ kg^{-1}$ soil	-	-	H	L	H	L
Fe	$mg\ kg^{-1}$ soil	H	L	H	L	H	L
Zn	$mg\ kg^{-1}$ soil	H	L	H	L	H	L
Mn	$mg\ kg^{-1}$ soil	H	L	H	L	H	L

† Suppr = Suppressive soil ‡ Cond = Conducive soil

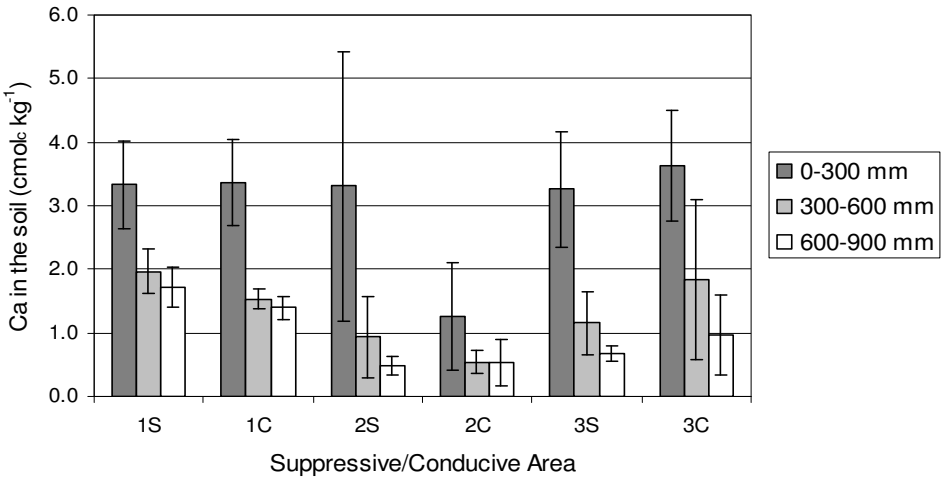

FIGURE 4.1. Average Ca ($cmol_c\ kg^{-1}$), extracted with a 0.02 M di-ammonium EDTA solution, at different sampling depths in suppressive (S) and conducive (C) soils of three banana fields (1, 2 and 3) in Kiepersol, South Africa. Bars indicate the standard deviation of 10 samples taken 0-300 mm in soils and 5 samples taken at depths of 300-600 and 600-900 mm.

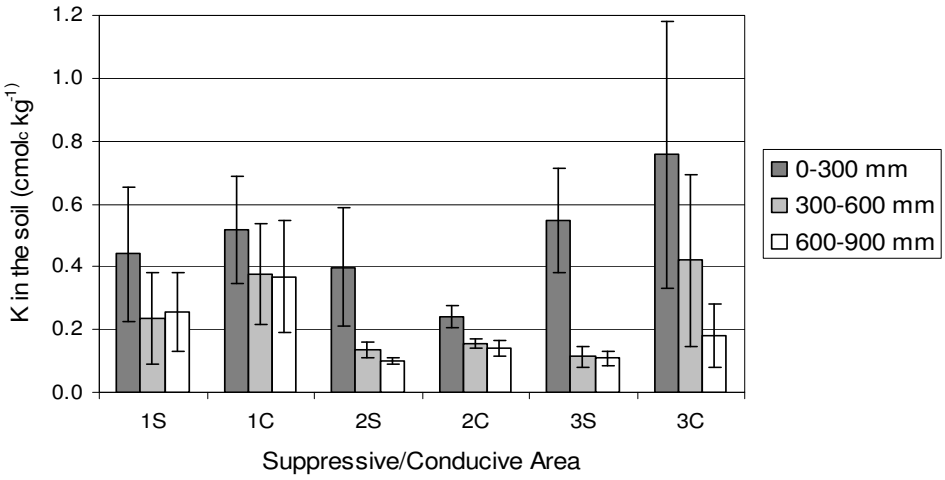

FIGURE 4.2. Average K ($cmol_c\ kg^{-1}$), extracted with a 0.02 M di-ammonium EDTA solution, at three sampling depths in suppressive (S) and conducive (C) soils of three banana fields (1, 2 and 3) in Kiepersol, South Africa. Bars indicate the standard deviation of 10 samples taken 0-300 mm in soils and 5 samples taken at depths of 300-600 and 600-900 mm.

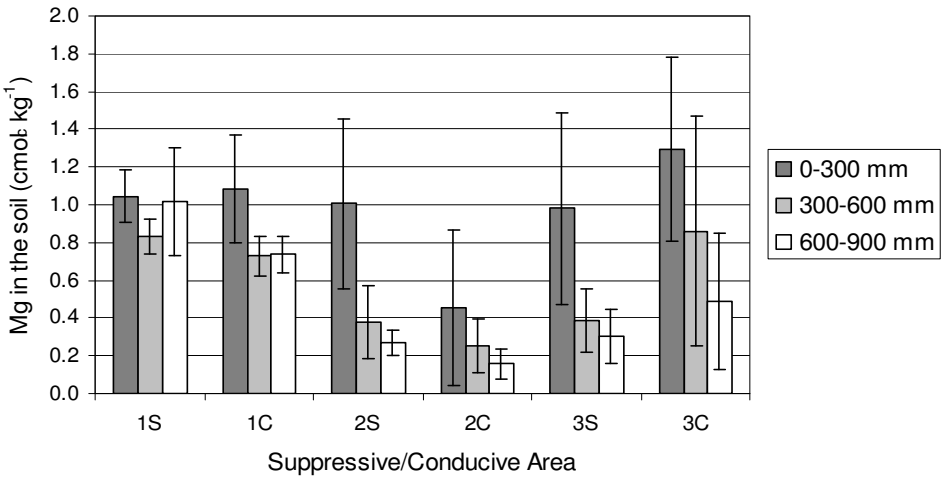

FIGURE 4.3. Average Mg ($cmol_c$ kg^{-1}), extracted with a 0.02 M di-ammonium EDTA solution, at different sampling depths in suppressive (S) and conducive (C) soils of three banana fields (1, 2 and 3) in Kiepersol, South Africa. Bars indicate the standard deviation of 10 samples taken 0-300 mm in soils and 5 samples taken at depths of 300-600 and 600-900 mm.

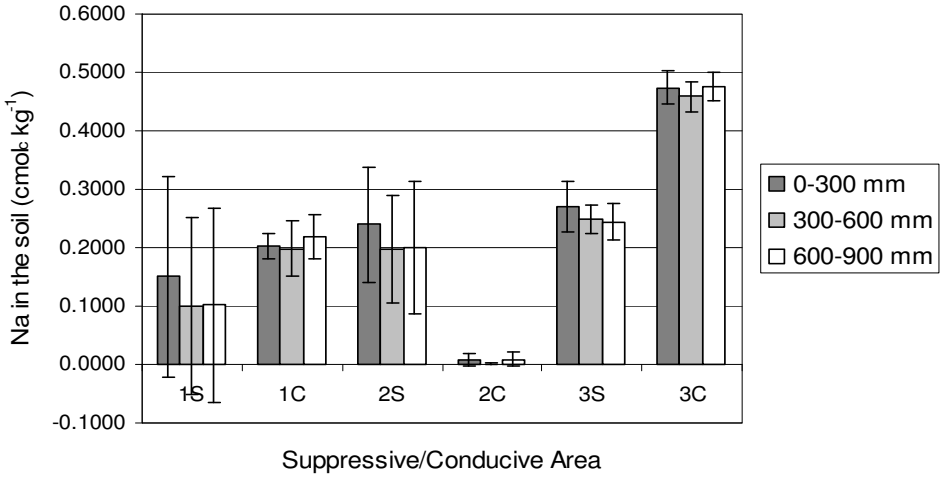

FIGURE 4.4. Average Na ($cmol_c$ kg^{-1}), extracted with a 0.02 M di-ammonium EDTA solution, at different sampling depths in suppressive (S) and conducive (C) soils of three banana fields (1, 2 and 3) in Kiepersol, South Africa. Bars indicate the standard deviation of 10 samples taken 0-300 mm in soils and 5 samples taken at depths of 300-600 and 600-900 mm.

The Na-content yielded differences between the suppressive and conducive soils at all sampling depths, with significant differences at all depths in Fields 2 and 3. In Fields 1 and 3 the Na-content was lower in the suppressive soils, which shows the same trend as that of the Ca, K and Mg. In Field 2 the trend was again reversed with significantly higher Na-levels in the suppressive soil, which supports the results of international researchers (Domínguez *et al.*, 1996, 2001; Peng *et al.*, 1999).

The variation in the amount of P in the three fields was high (Figure 4.5). In Field 1, the P in the soil was significantly lower in the suppressive soil compared to the conducive soil at the two upper sampling depths. Fields 2 and 3 showed an opposite trend with significantly higher P in the suppressive soils than in the conducive soils at the two higher sampling depths.

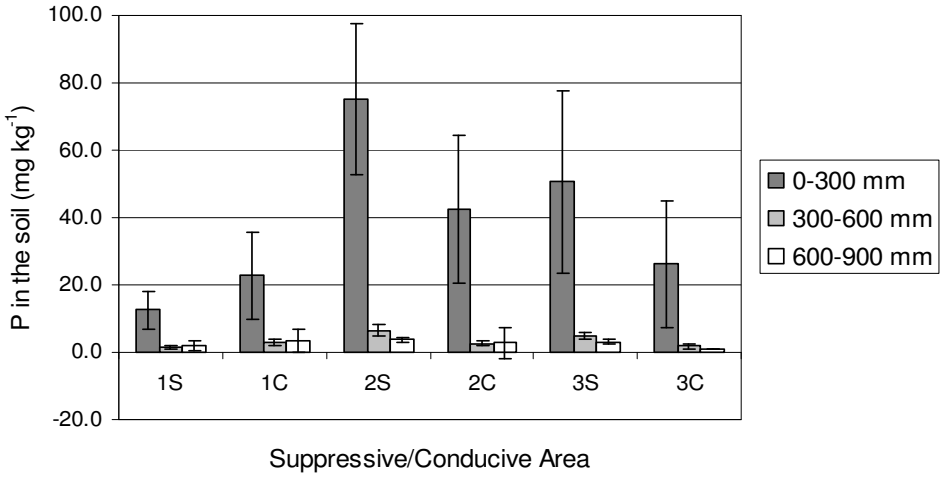

FIGURE 4.5. Average P (mg kg^{-1}), extracted with a Bray-I solution, at different sampling depths in suppressive (S) and conducive (C) soils of three banana fields (1, 2 and 3) in Kiepersol, South Africa. Bars indicate the standard deviation of 10 samples taken 0-300 mm in soils and 5 samples taken at depths of 300-600 and 600-900 mm.

DETERMINATION OF PH

In Fields 1 and 3 the pH(H_2O) was lower in the suppressive than in the conducive soils, with significance in the 0-30mm sampling layer (Figure 4.6). This is the opposite of the results of Peng *et al.* (1999) who found that the pH of a suppressive soil was higher than that of a conducive soil. The results in Field 2, on the other hand, correlate with those of Peng *et al.*

(1999) where the pH in the suppressive soil was slightly higher than in the conducive soil, with significance in the 0-30mm sampling layer.

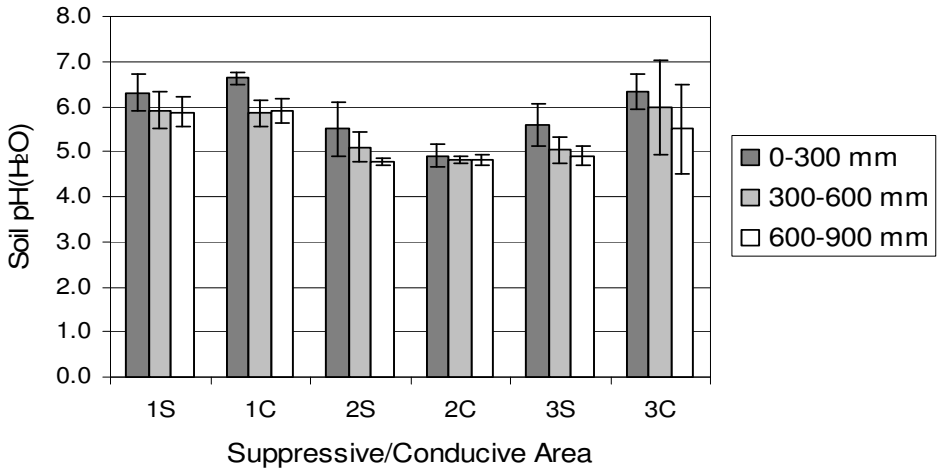

FIGURE 4.6. Average pH(H_2O) at different sampling depths of suppressive (S) and conducive (C) soils of three banana fields (1, 2 and 3) in Kiepersol, South Africa. Bars indicate the standard deviation of 10 samples taken 0-300 mm in soils and 5 samples taken at depths of 300-600 and 600-900 mm.

DETERMINATION OF N-CONTENT

A marked variation between the fields and soil depths existed in the NO_3^- and NH_4^+-values obtained for the three fields. The amount of nitrogen is influenced by the dynamic nature of nitrogen in soils. NO_3^- is very mobile in soils and can be leached beyond the rooting depth of plants, is subject to denitrification and volatilisation under the right conditions, and can be immobilised by microorganisms. NH_4^+ is much less mobile, but it can be rapidly converted to NO_3^- if conditions are favourable for nitrification (Alexander, 1961). Denitrification and volatilisation can occur after soil sampling, which can affect the eventual analysis of nitrogen content in the soil. The results obtained for the determination of nitrogen in the soil should therefore be viewed in this light.

Despite the dynamic nature of nitrogen, marked differences in N-content were found between the suppressive and conducive soils. In Field 1 the amount of nitrogen both in the form of NO_3^- and NH_4^+ was generally slightly lower in the suppressive than in the conducive soil (Figures 4.7 and 4.8). In Fields 2 and 3 the opposite was generally true, with higher NO_3^- and

NH_4^+-levels in the suppressive soils. The results from Field 1 in terms of NH_4^+-content therefore correlate with that of other researchers who indicated that higher levels of NH_4^+ were found in diseased soils (Domínguez *et al.*, 1996).

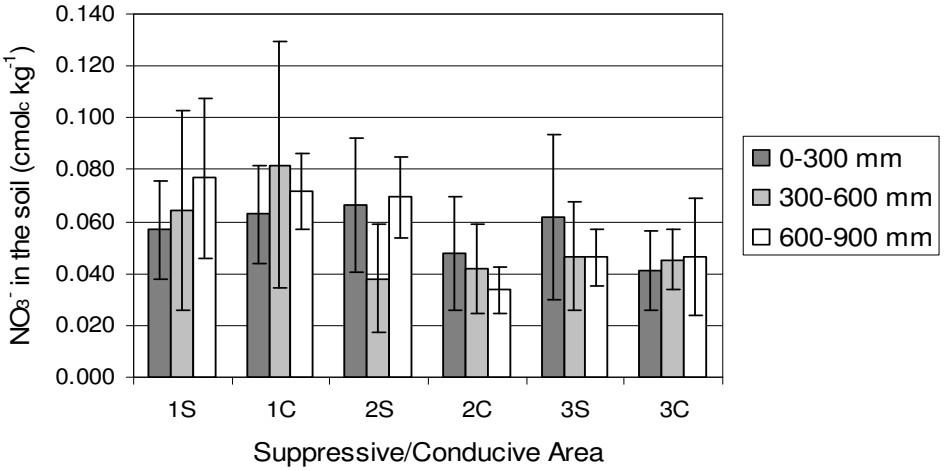

FIGURE 4.7. Average NO_3^- ($cmol_c\ kg^{-1}$), extracted with a 1 N solution of KCl, at different sampling depths in suppressive (S) and conducive (C) soils of three banana fields (1, 2 and 3) in Kiepersol, South Africa. Bars indicate the standard deviation of 10 samples taken 0-300 mm in soils and 5 samples taken at depths of 300-600 and 600-900 mm.

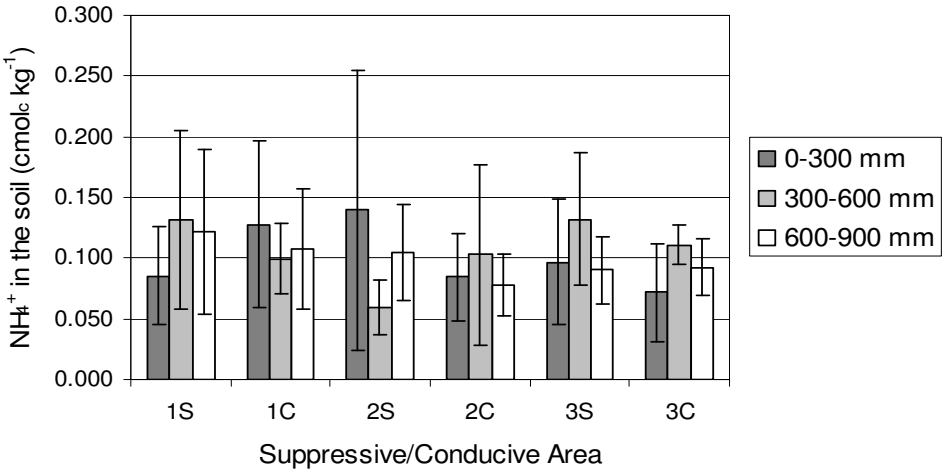

FIGURE 4.8. Average NH_4^+ ($cmol_c\ kg^{-1}$), extracted with a 1 N solution of KCl, at different sampling depths in suppressive (S) and conducive (C) soils of three banana fields (1, 2 and 3) in Kiepersol, South Africa. Bars indicate the standard deviation of 10 samples taken 0-300 mm in soils and 5 samples taken at depths of 300-600 and 600-900 mm.

DETERMINATION OF MICRO NUTRIENTS

The only consistent trend that was found in all three fields in the 0-300 mm sampling layer was in the levels of micro nutrients in the soil. The suppressive soils contained higher amounts of Cu (Figure 4.9), Fe (Figure 4.10), Zn (Figure 4.11) and Mn (Figure 4.12) compared to the conducive soils. These differences were significant in Fields 2 and 3, with the exception of the Cu-levels in Field 2. There was therefore a general trend of higher micro nutrients in the suppressive soils compared to the conducive soils. These results are unexpected, since previous researchers have found an increase in Fe, Mn and Zn to increase both disease incidence and -severity (Jones & Woltz, 1970; Peng *et al.*, 1999). The results in terms of Fe-content also do not correlate with results found in the literature in which Fe was higher in conducive soils than in suppressive soils (Domínguez *et al.*, 2001). These results warrant further investigations in the field to determine whether micro nutrients can be used in Kiepersol to manage the incidence of Fusarium wilt of banana.

Contrary to that of the 0-300 mm soil layer, the Mn in the two deeper soil layers of all the fields was higher in the conducive soils than in the suppressive soils, with significance in Field 3 (Figure 4.11). This is an indication of possible short periods of poor aeration and mineralisation of organic material in these deeper soil layers, since Mn is the first element amongst Mn, Cu, Fe and Zn to become soluble under such anaerobic conditions (Lindsay, 1979). This indicates that the deeper layers of the conducive soils are possibly more anaerobic and therefore contain more water than the suppressive soils. The possibility of over-irrigation in these conducive areas needs to be investigated and whether such over application of water creates conducive, anaerobic conditions by negatively affecting plant growth in the deeper soil layers.

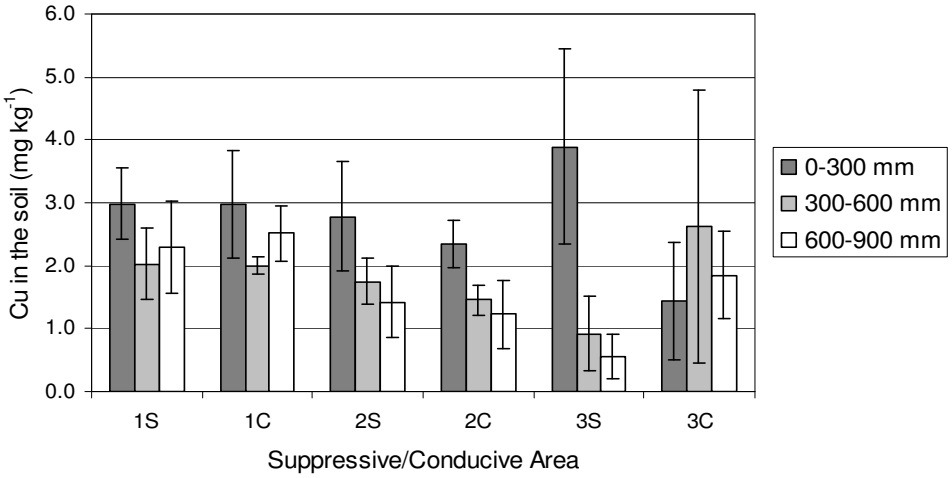

FIGURE 4.9. Average Cu (mg kg^{-1}), extracted with a 0.02 M di-ammonium EDTA solution, at different sampling depths in suppressive (S) and conducive (C) soils of three banana fields (1, 2 and 3) in Kiepersol, South Africa. Bars indicate the standard deviation of 10 samples taken 0-300 mm in soils and five samples taken at depths of 300-600 and 600-900 mm.

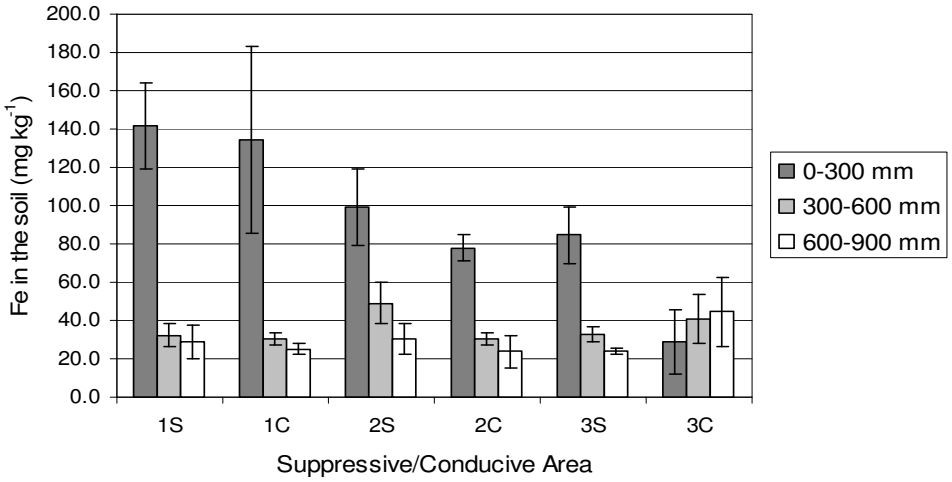

FIGURE 4.10. Average Fe (mg kg^{-1}), extracted with a 0.02 M di-ammonium EDTA solution, at different sampling depths in suppressive (S) and conducive (C) soils of three banana fields (1, 2 and 3) in Kiepersol, South Africa. Bars indicate the standard deviation of 10 samples taken 0-300 mm in soils and five samples taken at depths of 300-600 and 600-900 mm.

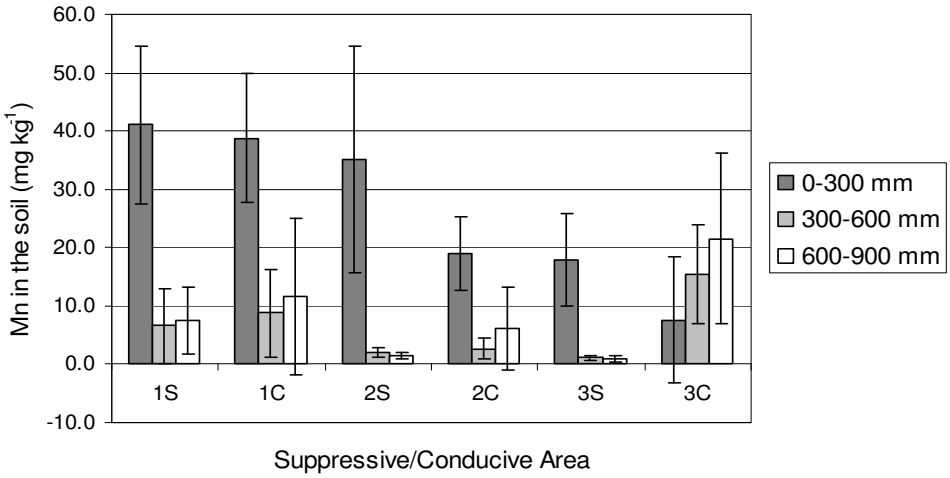

FIGURE 4.11. Average Mn (mg kg^{-1}), extracted with a 0.02 M di-ammonium EDTA solution, at different sampling depths in suppressive (S) and conducive (C) soils of three banana fields (1, 2 and 3) in Kiepersol, South Africa. Bars indicate the standard deviation of 10 samples taken 0-300 mm in soils and five samples taken at depths of 300-600 and 600-900 mm.

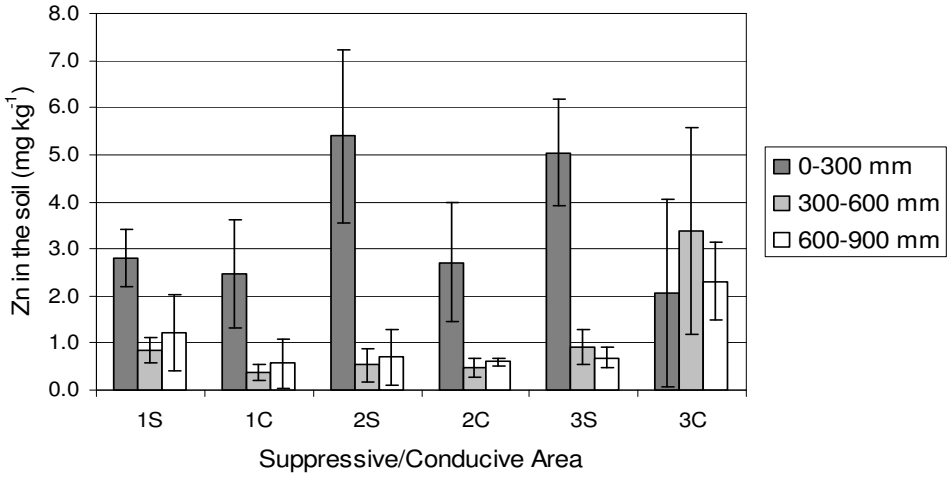

FIGURE 4.12. Average Zn (mg kg^{-1}), extracted with a 0.02 M di-ammonium EDTA solution, at different sampling depths in suppressive (S) and conducive (C) soils of three banana fields (1, 2 and 3) in Kiepersol, South Africa. Bars indicate the standard deviation of 10 samples taken 0-300 mm in soils and five samples taken at depths of 300-600 and 600-900 mm.

CONCLUSIONS

The results in this study provided clear soil chemical differences between soils suppressive and conducive to Fusarium wilt of banana. These differences, however, were not consistent in the three sampling fields. This reiterates the fact that differences in soil composition occur over very small distances. Each sampling field has its own composition and its own management practices, and needs to be evaluated according to its own soil properties. Trends found in one area cannot be extrapolated to another area with different soil chemical, physical and biological characteristics. A general field management strategy for Fusarium wilt of banana using macro and micro nutrients can therefore not be deduced from this study.

This study suggests that external factors, such as soil management practices, may have influenced the differences observed between suppressive and conducive soils. The application of chicken manure in the suppressive area of Field 2 could have been responsible for some of the inconsistencies between the chemical composition of suppressive and conducive areas in this field and the other two fields. The addition of this chicken manure could also be directly or indirectly responsible for the suppressiveness of this soil, as it would enhance the soil microbial activity. Analysis of the chicken manure, and field studies with the application thereof, are needed to determine its contribution to the observed soil properties and even its possible use in other areas. The management strategy in Fields 1 and 3 should also be investigated in order to find possible contributions to the observed results that may be applied in future studies.

Soil macro nutrients such as Ca, K, Mg, Na, and NO_3^- warrant proper investigation when studying suppressive and conducive soils, since the application of these elements in fertilisers in certain fields might influence data sets. The results from such an investigation will form the basis for further studies and will shed light on the true chemical composition of the soil. This information can then be used to determine which field trials should be conducted with different nutrient applications to determine their effect on Fusarium wilt development.

Due to the consistency of micro nutrient composition (Cu, Fe, Mn and especially Zn) of the three fields, it is proposed that further studies be conducted to determine the effect of changes in micro nutrient application on Fusarium wilt development. It has been stated that the pathogen is more sensitive to micro nutrient deficiencies than the host plant due to the

adaptation of plants to extract micro nutrients over greater distances (Woltz & Jones, 1981). However, in this study the levels of micro nutrients were higher in the suppressive soils, which indicate that the effect of micro nutrients on soil suppressiveness in these soils could not be attributed to the nutritional needs of the pathogen. It is therefore suggested that the interaction between these nutrients and the pathogen, and the nutrients and the plants be investigated in order to determine whether micro nutrient management can be used as part of an integrated management strategy to control Fusarium wilt of banana in South Africa.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

Although the banana plants in the pot trials were infected with *Foc*, the treatments of lime and nitrogen interacted to significantly affect the growth of the plants. In the soil medium, the optimum level of nitrogen application and nitrogen source was dependent on the addition of lime. Without the addition of lime to the soil, the best plant growth was achieved with low nitrogen application and at a low NO_3^-:NH_4^+ ratio. In other words, when no lime is added, NH_4^+-N is essential for better plant growth in the presence of Fusarium wilt. At the other end of the spectrum, when lime was added to the soil, the best plant growth required high nitrogen applications, mostly in the form of NO_3^-. NH_4^+-N was not beneficial to plant growth when accompanied by liming. The latter results correspond with those found by Woltz and Jones (1981) who indicated that low NH_4^+-N and high NO_3^--N application was more favourable to plants in reducing the development of Fusarium wilt. The interaction between the lime and the NO_3^--N was suggested to raise the soil pH and thereby decreases the pathogenicity of *Fusarium oxysporum*, while the addition of NH_4^+-N decreases the soil and rhizosphere pH (Engelhard, 1989). This increase in pH through liming and nitrate application could also affect the pathogen due to a lowered concentration of nutrients such as phosphorous, magnesium, sulphur and possibly copper in the soil (Woltz & Jones, 1981).

The results in terms of nitrogen uptake by the plants mirrored the results in terms of plant growth and indicate that effective N-uptake is essential for better plant growth in the presence of Fusarium wilt. This also indicates that effective nitrogen fertilisation can be used to improve plant growth in spite of the presence of *Foc* and that high nitrogen application does not necessarily correlate with high nitrogen uptake by plants.

In the hydroponic trial, a change in pH had a significant effect on plant growth, with the lower pH resulting in lower plant growth compared to the higher pH in plants infected with *Foc*. The opposite was found in the control treatments with higher plant growth in the lower pH treatments, which indicates that infection of plants by *Foc* changes the pH- and nutritional requirements of the plants in terms of nitrogen. A lower nitrogen application was generally better for plant growth, but the source of nitrogen did not have a profound effect. The effect of nitrogen treatments on plant growth was therefore not as significant as in the soil pot trial.

These results support the suggestion in previous research that the rhizosphere in the soil has a role to play in the effect that liming or pH and nitrogen treatment has on plants infected with Fusarium wilt (Engelhard, 1989). The rhizosphere and the effect of its chemistry on Fusarium wilt and specifically *Foc* should be investigated and quantified in further studies.

Analysis of the three field soils indicated that any soil analysis done in a specific area or soil cannot be applied or extrapolated to another area. The only consistent trend that was found in all three the fields was that the micro nutrients Cu, Fe, Mn and Zn were higher in the suppressive soils than in the conducive soils. Trends that were observed in terms of the macro nutrients, nitrogen and pH varied between the three fields. Although it has been indicated that the pathogen is more sensitive to micro nutrient deficiencies than the host plant (Woltz & Jones, 1981), the micro nutrient levels in these soils are higher in the suppressive soils. The possible effect that these micro nutrients have on Fusarium wilt development is therefore due to a different interaction between the nutrients and the pathogen, and/or the nutrients and the plants. These interactions need to be studied and evaluated in further trials. It is also suggested that the external factors that contribute to the soil environment, such as management practices and fertilisation programs, be investigated to determine how these factors contribute to the suppressive or conducive nature of the respective soils.

Overall, it was concluded that Fusarium wilt of banana and the growth of plants infected therewith is affected by liming, pH and nitrogen fertilisation. It is also concluded that measurable differences occur in the chemical properties of soils suppressive and conducive to Fusarium wilt. Further studies are needed in specific fields in Kiepersol (Mpumalanga) where Fusarium wilt occurs, to investigate the effect of liming and nitrogen fertilisation in the field on Fusarium wilt, plant growth and also on the chemical properties in the soil. The nutritional requirements of banana plants under these conditions need to be determined in order to provide the plants with the optimal growing conditions and give the pathogen the most resistance. The goal is to create an integrated management strategy to best control Fusarium wilt of banana in order to enable the continued production of bananas in Kiepersol.

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APPENDIX A.

Volume (ml) of each stock solution added per pot (containing 4 kg of soil) every 3 weeks in the soil pot trial

		N1 (0.27 mmol N pot^{-1})			N2 (0.55 mmol N pot^{-1})			N3 (0.82 mmol N pot^{-1})		
	Control	V1(9:6)	V2(11:4)	V3(14:1)	V1(9:6)	V2(11:4)	V3(14:1)	V1(9:6)	V2(11:4)	V3(14:1)
0.01 M Solution										
NH_4NO_3	0	44	29.3	7.3	88	58.7	14.7	132	88	22
KNO_3	0	22	51.4	95.4	132	102.6	190.6	66	154	286

APPENDIX B.

Volume (ml) of each stock solution needed to make up 1 L of nutrient solution for the low pH treatments in the hydroponic pot trial every 3 weeks

		Low pH (pH 6)								
		N1 (0.27 mmol N pot^{-1})			N2 (0.55 mmol N pot^{-1})			N3 (0.82 mmol N pot^{-1})		
1 M Solution	Control	V1(9:6)	V2(11:4)	V3(14:1)	V1(9:6)	V2(11:4)	V3(14:1)	V1(9:6)	V2(11:4)	V3(14:1)
KH_2PO_4		1	1	1	1	1		1		
$NH_4H_2PO_4$	1						1		1	1
KNO_3	6			1	1	3	6	5	6	6
$MgSO_4$	2	2	2	2	2	2	2	2	1	
$Ca(NO_3)_2$	4	2	2	3	4	4	4	4	4	5
$Mg(NO_3)_2$									1	2
$(NH_4)_2SO_4$		1.25	0.25	0.25	3	2		4.25	2.25	
NH_4NO_3		0.5	1.5					0.5	0.5	0.5
$KHCO_3$	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
0.5 M $CLSO_4$		5	5	4	4	2				
$CaSO_4.2H_2O$		0.308 g L^{-1}	0.308 g L^{-1}	0.154 g L^{-1}						
Micro elements										
$FeSO_4.7H_2O$	1	1	1	1	1	1	1	1	1	1
$MnSO_4.H_2O$	1	1	1	1	1	1	1	1	1	1
$CuSO_4.5H_2O$	1	1	1	1	1	1	1	1	1	1
$ZnSO_4.7H_2O$	1	1	1	1	1	1	1	1	1	1
H_3BO_3	1	1	1	1	1	1	1	1	1	1
$Na_2MoO_4.2H_2O$	1	1	1	1	1	1	1	1	1	1

APPENDIX C.

Volume (ml) of each stock solution needed to make up 1 L of nutrient solution for the high pH treatments in the hydroponic pot trial every 3 weeks

		High pH (pH 7)								
		N1 (0.27 mmol N pot^{-1})			N2 (0.55 mmol N pot^{-1})			N3 (0.82 mmol N pot^{-1})		
1 M Solution	Control	V1(9:6)	V2(11:4)	V3(14:1)	V1(9:6)	V2(11:4)	V3(14:1)	V1(9:6)	V2(11:4)	V3(14:1)
KH_2PO_4		1	1	1	1	1		1		
$NH_4H_2PO_4$	1						1		1	1
KNO_3	2			1	1	3	2	2	3	3
$MgSO_4$		2	2	2	2	2		0.5		
$Ca(NO_3)_2$	4	2	2	3	4	4	4	4	4	5.5
$Mg(NO_3)_2$	2						2	1.5	2.5	3
$(NH_4)_2SO_4$		1.25	0.25	0.25	3	2		4.25	2.25	
NH_4NO_3		0.5	1.5					0.5	0.5	0.5
$KHCO_3$	3	3	3	3	3	3	3	3	3	3
0.5 M $CLSO_4$	1	2	2	1	1	0	1			
$CaSO_4.2H_2O$		0.308 g L^{-1}	0.308 g L^{-1}	0.154 g L^{-1}						
Micro elements										
$FeSO_4.7H_2O$	1	1	1	1	1	1	1	1	1	1
$MnSO_4.H_2O$	1	1	1	1	1	1	1	1	1	1
$CuSO_4.5H_2O$	1	1	1	1	1	1	1	1	1	1
$ZnSO_4.7H_2O$	1	1	1	1	1	1	1	1	1	1
H_3BO_3	1	1	1	1	1	1	1	1	1	1
$Na_2MoO_4.2H_2O$	1	1	1	1	1	1	1	1	1	1

APPENDIX D

Factorial Analysis of Variance of the above ground dry mass of banana plants grown in pots with soil in the greenhouse and infected with Fusarium wilt

Source of variation	DF	SS	MS	F	F.pr.	SS%
pH	1	0.2200	0.2200	0.30	0.586	0.35%
Nitrogen level (N)	2	1.9143	0.9572	1.31	0.279	3.07%
Nitrogen ratio (V)	2	0.2996	0.1498	0.20	0.0816	0.48%
pH.N	2	2.8969	1.4484	1.98	0.148	4.64%
pH.V	2	6.4856	3.2428	4.43	0.017	10.39%
N.V	4	7.2899	1.8225	2.49	0.054	11.68%
pH.N.V	4	3.8024	0.9506	1.30	0.282	3.09%
Residual	54	39.5161	0.7318			63.30%
Total	71	62.4247				

APPENDIX E

Factorial Analysis of Variance of the %N in the above ground dry material of banana plants grown in pots with soil in the greenhouse and infected with Fusarium wilt

Source of variation	DF	SS	MS	F	F.pr.	SS%
pH	1	0.11923	0.11923	3.44	0.069	3.13%
Nitrogen level (N)	2	0.42387	0.21193	6.12	0.004	11.12%
Nitrogen ratio (V)	2	0.01221	0.00611	0.18	0.839	0.32%
pH.N	2	0.68744	0.34372	9.92	<.001	18.04%
pH.V	2	0.11254	0.05627	1.62	0.207	2.95%
N.V	4	0.38611	0.09653	2.79	0.035	10.13%
pH.N.V	4	0.19770	0.04942	1.43	0.238	5.19%
Residual	54	1.87153	0.03466			49.11%
Total	71	3.81063				

APPENDIX F

Factorial Analysis of Variance of the above ground dry mass of banana plants grown in hydroponic pots in the greenhouse and infected with Fusarium wilt

Source of variation	DF	SS	MS	F	F.pr.	SS%
Nitrogen level (N)	2	572.37	286.18	11.45	<.001	16.95%
pH	1	887.26	887.26	35.51	<.001	26.27%
Nitrogen ratio (V)	2	15.16	7.58	0.30	0.740	0.45%
N.pH	2	46.29	23.15	0.93	0.402	1.37%
N.V	4	186.09	46.52	1.86	0.131	5.51%
pH.V	2	87.85	43.93	1.76	0.182	2.60%
N.pH.V	4	233.30	58.33	2.33	0.067	6.91%
Residual	54	1349.22	24.99			39.95%
Total	71	3377.54				

APPENDIX G

Factorial Analysis of Variance of the %N in the above ground dry material of banana plants grown in hydroponic pots in the greenhouse and infected with Fusarium wilt

Source of variation	DF	SS	MS	F	F.pr.	SS%
Nitrogen level (N)	2	6.31977	3.15988	42.52	<.001	37.23
pH	1	0.53389	0.53389	7.18	0.010	3.15
Nitrogen ratio (V)	2	1.26195	0.63098	8.49	<.001	7.43
N.pH	2	3.53547	1.76773	23.79	<.001	20.83
N.V	4	0.12671	0.03168	0.43	0.789	0.75
pH.V	2	0.03127	0.01563	0.21	0.811	0.18
N.pH.V	4	1.15250	0.28812	3.88	0.008	6.79
Residual	54	4.01270	0.07431			23.64
Total	71	16.97426				

APPENDIX H.

Raw data of the analysis of suppressive and conducive soils from three fields in Kiepersol, South Africa

Sample	Depth (mm)	Suppr/Cond	P (mg kg^{-1})	Ca ($cmol_c$ kg^{-1})	K ($cmol_c$ kg^{-1})	Mg ($cmol_c$ kg^{-1})	Na ($cmol_c$ kg^{-1})	pH(H_2O)	pH(KCl)
S1	0-300	Suppr	18.2	4.04212	0.49875	0.97922	0.09134	6.35	5.54
S1	0-300	Suppr	9.5	3.38839	0.37854	1.10265	0.00870	6.30	5.42
S1	0-300	Suppr	23.1	3.07401	0.71615	1.01214	0.00000	6.25	5.38
S1	0-300	Suppr	9.8	2.83946	0.32994	1.02037	0.03480	6.40	5.59
S1	0-300	Suppr	3.1	3.36843	0.20717	0.88871	0.04785	6.31	5.47
S1	0-300	Suppr	8.4	3.74270	0.23531	1.29192	0.03915	6.47	5.71
S1	0-300	Suppr	12.5	4.33155	0.40667	1.18494	0.09569	6.57	5.78
S1	0-300	Suppr	15.9	3.86247	0.88751	1.17671	0.37843	7.05	6.05
S1	0-300	Suppr	11.3	2.32547	0.43736	0.91339	0.41757	5.51	4.68
S1	0-300	Suppr	13.6	2.35541	0.30181	0.88048	0.38712	5.86	4.87
C1	0-300	Cond	11.8	2.73966	0.36063	0.78996	0.23054	6.31	5.43
C1	0-300	Cond	14.5	3.34348	0.41179	0.95454	0.21314	6.50	5.82
C1	0-300	Cond	14.4	2.99915	0.39900	0.91339	0.20444	6.84	6.10
C1	0-300	Cond	52.6	2.80952	0.79544	1.10265	0.18269	6.61	5.89
C1	0-300	Cond	34	4.36150	0.74428	1.63752	0.22184	6.66	5.78
C1	0-300	Cond	18.4	3.81257	0.41690	1.46472	0.19139	6.56	5.81
C1	0-300	Cond	12.9	3.36843	0.27879	0.96276	0.20009	6.71	5.81
C1	0-300	Cond	29.2	4.12695	0.62919	1.06974	0.23054	6.65	5.82
C1	0-300	Cond	26.1	3.84251	0.52177	1.23431	0.17834	6.74	5.96
C1	0-300	Cond	15.2	2.23564	0.61640	0.74059	0.16964	6.67	5.42

Raw data of the analysis of suppressive and conducive soils from three fields in Kiepersol, South Africa

Sample	Depth (mm)	Suppr/Cond	P ($mg\ kg^{-1}$)	Ca ($cmol_c\ kg^{-1}$)	K ($cmol_c\ kg^{-1}$)	Mg ($cmol_c\ kg^{-1}$)	Na ($cmol_c\ kg^{-1}$)	pH(H_2O)	pH(KCl)
S1	300-600	Suppr	1.3	2.17576	0.15602	0.74882	0.00000	5.87	5.26
S1	300-600	Suppr	2.1	1.88133	0.28902	0.84756	0.00000	5.93	5.29
S1	300-600	Suppr	1.0	2.20570	0.04348	0.97922	0.05220	6.09	5.44
S1	300-600	Suppr	1.0	2.17576	0.42713	0.83933	0.08699	6.40	5.68
S1	300-600	Suppr	1.0	1.40227	0.26088	0.75705	0.36103	5.28	4.48
C1	300-600	Cond	2.2	1.41724	0.35296	0.74059	0.23054	5.52	4.99
C1	300-600	Cond	3.9	1.32741	0.61128	0.68299	0.21314	6.15	5.59
C1	300-600	Cond	2.1	1.73162	0.31204	0.87225	0.22619	6.02	5.38
C1	300-600	Cond	2.7	1.64679	0.43480	0.75705	0.20444	6.01	5.50
C1	300-600	Cond	3.6	1.55197	0.17648	0.58424	0.11744	5.58	5.05
S1	600-900	Suppr	1.4	1.68671	0.17136	0.88048	0.00261	5.76	5.28
S1	600-900	Suppr	1.0	1.52702	0.27367	0.85579	0.01305	5.82	5.23
S1	600-900	Suppr	4.4	2.26059	0.15346	1.52232	0.06090	6.19	5.60
S1	600-900	Suppr	1.2	1.52203	0.46550	0.85579	0.39582	6.22	5.60
S1	600-900	Suppr	1.0	1.54698	0.22252	0.97922	0.03480	5.42	4.74
C1	600-900	Cond	1.2	1.25755	0.35296	0.65830	0.20879	5.86	5.55
C1	600-900	Cond	9.5	1.71665	0.67778	0.76527	0.26533	6.20	5.63
C1	600-900	Cond	1.9	1.27252	0.32994	0.82288	0.25228	6.07	5.48
C1	600-900	Cond	2	1.38230	0.24042	0.82288	0.19139	5.97	5.52
C1	600-900	Cond	2.8	1.34238	0.24298	0.61716	0.17834	5.49	5.05

Raw data of the analysis of suppressive and conducive soils from three fields in Kiepersol, South Africa

Sample	Depth (mm)	Suppr/Cond	NH_4 ($cmol_c\ kg^{-1}$)	NO_3 ($cmol_c\ kg^{-1}$)	Cu ($mg\ kg^{-1}$)	Fe ($mg\ kg^{-1}$)	Mn ($mg\ kg^{-1}$)	Zn ($mg\ kg^{-1}$)
S1	0-300	Suppr	0.04284	0.06105	2.84	144.23	44.28	3.02
S1	0-300	Suppr	0.14715	0.05130	3.78	133.65	39.56	3.51
S1	0-300	Suppr	0.07513	0.08345	2.25	112.73	26.28	3.47
S1	0-300	Suppr	0.14467	0.06322	2.79	117.68	39.78	2.79
S1	0-300	Suppr	0.04408	0.03829	3.96	144.90	47.88	1.71
S1	0-300	Suppr	0.09748	0.06647	2.52	156.15	37.98	2.30
S1	0-300	Suppr	0.03850	0.03920	3.24	188.33	64.31	3.33
S1	0-300	Suppr	0.05816	0.03257	3.29	160.20	58.46	3.15
S1	0-300	Suppr	0.11238	0.08779	2.57	130.50	31.46	2.30
S1	0-300	Suppr	0.09500	0.04444	2.61	129.15	20.43	2.48
C1	0-300	Cond	0.06768	0.04696	2.48	149.13	49.05	3.74
C1	0-300	Cond	0.05526	0.05166	2.57	106.16	27.86	1.22
C1	0-300	Cond	0.07761	0.05383	3.02	120.56	39.78	2.21
C1	0-300	Cond	0.13722	0.08201	2.66	125.96	28.53	3.69
C1	0-300	Cond	0.14839	0.08020	4.77	233.96	45.18	4.32
C1	0-300	Cond	0.12728	0.07225	2.88	146.43	50.81	1.76
C1	0-300	Cond	0.05278	0.09899	3.83	159.71	50.81	2.30
C1	0-300	Cond	0.15709	0.04480	3.33	150.71	42.26	2.16
C1	0-300	Cond	0.18192	0.04660	2.52	113.58	34.83	2.48
C1	0-300	Cond	0.27161	0.05058	1.67	40.14	18.41	0.77

Raw data of the analysis of suppressive and conducive soils from three fields in Kiepersol, South Africa

Sample	Depth (mm)	Suppr/Cond	NH_4 ($cmol_c\ kg^{-1}$)	NO_3 ($cmol_c\ kg^{-1}$)	Cu ($mg\ kg^{-1}$)	Fe ($mg\ kg^{-1}$)	Mn ($mg\ kg^{-1}$)	Zn ($mg\ kg^{-1}$)
S1	300-600	Suppr	0.09624	0.03685	1.76	27.54	2.97	0.72
S1	300-600	Suppr	0.16733	0.09610	1.71	26.60	5.90	1.13
S1	300-600	Suppr	0.06023	0.04100	3.02	41.85	17.51	1.17
S1	300-600	Suppr	0.24339	0.11552	1.80	33.44	4.37	0.59
S1	300-600	Suppr	0.09282	0.03242	1.85	31.10	1.98	0.68
C1	300-600	Cond	0.09282	0.07939	1.98	29.16	3.33	0.23
C1	300-600	Cond	0.09593	0.05545	2.12	33.44	5.63	0.59
C1	300-600	Cond	0.15026	0.07668	1.98	33.44	7.34	0.27
C1	300-600	Cond	0.07758	0.16067	1.80	25.47	5.67	0.23
C1	300-600	Cond	0.08196	0.03694	2.12	30.96	22.01	0.50
S1	600-900	Suppr	0.08817	0.09790	1.89	22.50	3.33	0.72
S1	600-900	Suppr	0.06954	0.10513	2.07	22.41	4.91	1.17
S1	600-900	Suppr	0.20614	0.05184	3.51	38.93	7.70	2.61
S1	600-900	Suppr	0.06271	0.03540	1.62	21.92	4.68	0.59
S1	600-900	Suppr	0.18440	0.09339	2.39	38.03	17.51	0.99
C1	600-900	Cond	0.09438	0.09384	2.16	23.40	4.01	0.18
C1	600-900	Cond	0.08041	0.07081	2.88	23.99	8.19	1.40
C1	600-900	Cond	0.09438	0.05862	1.98	23.99	4.95	0.23
C1	600-900	Cond	0.07420	0.05907	2.97	24.03	5.67	0.72
C1	600-900	Cond	0.19527	0.07713	2.57	30.38	35.50	0.27

Raw data of the analysis of suppressive and conducive soils from three fields in Kiepersol, South Africa

Sample	Depth (mm)	Suppr/Cond	P (mg kg^{-1})	Ca ($cmol_c$ kg^{-1})	K ($cmol_c$ kg^{-1})	Mg ($cmol_c$ kg^{-1})	Na ($cmol_c$ kg^{-1})	pH(H_2O)	pH(KCl)
S2	0-300	Suppr	83.9	7.37562	0.30436	1.65398	0.34798	6.16	5.24
S2	0-300	Suppr	85	4.10699	0.31204	1.20963	0.39582	5.71	4.94
S2	0-300	Suppr	47.4	3.39338	0.31459	1.20963	0.38712	5.84	4.89
S2	0-300	Suppr	64.4	2.88937	0.92332	1.44003	0.18704	5.64	5.12
S2	0-300	Suppr	53.8	2.99915	0.34529	0.88048	0.16964	5.39	4.59
S2	0-300	Suppr	110.1	2.19572	0.28134	0.78996	0.13049	5.24	4.49
S2	0-300	Suppr	113.2	6.12805	0.42713	1.31660	0.19574	6.26	5.28
S2	0-300	Suppr	69.3	2.35541	0.39132	0.91339	0.16094	5.64	4.86
S2	0-300	Suppr	66.3	1.05794	0.38109	0.43612	0.23923	4.82	4.02
S2	0-300	Suppr	57.8	0.56390	0.30692	0.21395	0.18269	4.31	3.87
C2	0-300	Cond	9.6	0.42916	0.22763	0.07406	0.00391	4.61	3.86
C2	0-300	Cond	21	0.80343	0.28134	0.18926	0.02175	4.82	3.98
C2	0-300	Cond	31.7	1.56195	0.21484	0.55133	0.01740	4.96	4.15
C2	0-300	Cond	79.5	1.14277	0.25577	0.38675	0.00174	5.02	4.00
C2	0-300	Cond	46.9	1.52203	0.19694	0.74882	0.00174	5.03	4.15
C2	0-300	Cond	26	0.63875	0.23275	0.10697	0.00000	4.61	3.89
C2	0-300	Cond	57.6	3.35845	0.29413	1.36597	0.00174	5.40	4.60
C2	0-300	Cond	36.8	0.75852	0.23786	0.18103	0.03045	4.95	3.96
C2	0-300	Cond	70	0.78347	0.27879	0.18103	0.00870	4.58	3.87
C2	0-300	Cond	46.9	1.52203	0.19694	0.74882	0.00174	5.03	4.15

Raw data of the analysis of suppressive and conducive soils from three fields in Kiepersol, South Africa

Sample	Depth (mm)	Suppr/Cond	P (mg kg^{-1})	Ca ($cmol_c$ kg^{-1})	K ($cmol_c$ kg^{-1})	Mg ($cmol_c$ kg^{-1})	Na ($cmol_c$ kg^{-1})	pH(H_2O)	pH(KCl)
S2	300-600	Suppr	6.2	0.88328	0.12533	0.46904	0.36103	5.37	4.19
S2	300-600	Suppr	4.5	0.64873	0.10742	0.31269	0.16964	5.03	4.17
S2	300-600	Suppr	5.8	0.66870	0.13556	0.28801	0.13484	4.91	4.16
S2	300-600	Suppr	8.4	2.04601	0.13044	0.65830	0.16094	5.52	4.57
S2	300-600	Suppr	7.7	0.41419	0.17904	0.16458	0.16529	4.69	4.05
C2	300-600	Cond	2.7	0.72858	0.15858	0.14812	0.00435	4.77	4.06
C2	300-600	Cond	3.9	0.70363	0.17392	0.17280	0.00000	4.94	4.10
C2	300-600	Cond	2	0.34932	0.13811	0.41144	0.00000	4.80	4.08
C2	300-600	Cond	2.1	0.54893	0.16625	0.12343	0.00000	4.73	4.04
C2	300-600	Cond	2	0.34932	0.13811	0.41144	0.00000	4.80	4.08
S2	600-900	Suppr	3.3	0.51899	0.10486	0.34561	0.40017	4.85	4.11
S2	600-900	Suppr	3.6	0.32437	0.08185	0.18926	0.12179	4.71	4.15
S2	600-900	Suppr	3	0.47907	0.11254	0.29624	0.14354	4.74	4.19
S2	600-900	Suppr	4	0.71361	0.09975	0.31269	0.15224	4.85	4.18
S2	600-900	Suppr	4.7	0.42916	0.09719	0.21395	0.18269	4.72	4.10
C2	600-900	Cond	10.9	1.03798	0.17392	0.24686	0.00870	4.88	4.12
C2	600-900	Cond	0.8	0.72359	0.14067	0.19749	0.03045	4.95	4.28
C2	600-900	Cond	0	0.17965	0.11510	0.07406	0.00217	4.71	4.10
C2	600-900	Cond	1.9	0.56889	0.15346	0.19749	0.00000	4.83	4.19
C2	600-900	Cond	0	0.17965	0.11510	0.07406	0.00217	4.71	4.10

Raw data of the analysis of suppressive and conducive soils from three fields in Kiepersol, South Africa

Sample	Depth (mm)	Suppr/Cond	NH_4 ($cmol_c\ kg^{-1}$)	NO_3 ($cmol_c\ kg^{-1}$)	Cu ($mg\ kg^{-1}$)	Fe ($mg\ kg^{-1}$)	Mn ($mg\ kg^{-1}$)	Zn ($mg\ kg^{-1}$)
S2	0-300	Suppr	0.04284	0.06105	2.84	144.23	44.28	3.02
S2	0-300	Suppr	0.14715	0.05130	3.78	133.65	39.56	3.51
S2	0-300	Suppr	0.07513	0.08345	2.25	112.73	26.28	3.47
S2	0-300	Suppr	0.14467	0.06322	2.79	117.68	39.78	2.79
S2	0-300	Suppr	0.04408	0.03829	3.96	144.90	47.88	1.71
S2	0-300	Suppr	0.09748	0.06647	2.52	156.15	37.98	2.30
S2	0-300	Suppr	0.03850	0.03920	3.24	188.33	64.31	3.33
S2	0-300	Suppr	0.05816	0.03257	3.29	160.20	58.46	3.15
S2	0-300	Suppr	0.11238	0.08779	2.57	130.50	31.46	2.30
S2	0-300	Suppr	0.09500	0.04444	2.61	129.15	20.43	2.48
C2	0-300	Cond	0.06768	0.04696	2.48	149.13	49.05	3.74
C2	0-300	Cond	0.05526	0.05166	2.57	106.16	27.86	1.22
C2	0-300	Cond	0.07761	0.05383	3.02	120.56	39.78	2.21
C2	0-300	Cond	0.13722	0.08201	2.66	125.96	28.53	3.69
C2	0-300	Cond	0.14839	0.08020	4.77	233.96	45.18	4.32
C2	0-300	Cond	0.12728	0.07225	2.88	146.43	50.81	1.76
C2	0-300	Cond	0.05278	0.09899	3.83	159.71	50.81	2.30
C2	0-300	Cond	0.15709	0.04480	3.33	150.71	42.26	2.16
C2	0-300	Cond	0.18192	0.04660	2.52	113.58	34.83	2.48
C2	0-300	Cond	0.27161	0.05058	1.67	40.14	18.41	0.77

Raw data of the analysis of suppressive and conducive soils from three fields in Kiepersol, South Africa

Sample	Depth (mm)	Suppr/Cond	NH_4 ($cmol_c\ kg^{-1}$)	NO_3 ($cmol_c\ kg^{-1}$)	Cu ($mg\ kg^{-1}$)	Fe ($mg\ kg^{-1}$)	Mn ($mg\ kg^{-1}$)	Zn ($mg\ kg^{-1}$)
S2	300-600	Suppr	0.09624	0.03685	1.76	27.54	2.97	0.72
S2	300-600	Suppr	0.16733	0.09610	1.71	26.60	5.90	1.13
S2	300-600	Suppr	0.06023	0.04100	3.02	41.85	17.51	1.17
S2	300-600	Suppr	0.24339	0.11552	1.80	33.44	4.37	0.59
S2	300-600	Suppr	0.09282	0.03242	1.85	31.10	1.98	0.68
C2	300-600	Cond	0.09282	0.07939	1.98	29.16	3.33	0.23
C2	300-600	Cond	0.09593	0.05545	2.12	33.44	5.63	0.59
C2	300-600	Cond	0.15026	0.07668	1.98	33.44	7.34	0.27
C2	300-600	Cond	0.07758	0.16067	1.80	25.47	5.67	0.23
C2	300-600	Cond	0.08196	0.03694	2.12	30.96	22.01	0.50
S2	600-900	Suppr	0.08817	0.09790	1.89	22.50	3.33	0.72
S2	600-900	Suppr	0.06954	0.10513	2.07	22.41	4.91	1.17
S2	600-900	Suppr	0.20614	0.05184	3.51	38.93	7.70	2.61
S2	600-900	Suppr	0.06271	0.03540	1.62	21.92	4.68	0.59
S2	600-900	Suppr	0.18440	0.09339	2.39	38.03	17.51	0.99
C2	600-900	Cond	0.09438	0.09384	2.16	23.40	4.01	0.18
C2	600-900	Cond	0.08041	0.07081	2.88	23.99	8.19	1.40
C2	600-900	Cond	0.09438	0.05862	1.98	23.99	4.95	0.23
C2	600-900	Cond	0.07420	0.05907	2.97	24.03	5.67	0.72
C2	600-900	Cond	0.19527	0.07713	2.57	30.38	35.50	0.27

Raw data of the analysis of suppressive and conducive soils from three fields in Kiepersol, South Africa

Sample	Depth (mm)	Suppr/Cond	P (mg kg^{-1})	Ca ($cmol_c$ kg^{-1})	K ($cmol_c$ kg^{-1})	Mg ($cmol_c$ kg^{-1})	Na ($cmol_c$ kg^{-1})	pH(H_2O)	pH(KCl)
S3	0-300	Suppr	86.5	4.73077	0.36831	1.37420	0.23054	5.98	5.39
S3	0-300	Suppr	44.3	2.69974	0.63686	0.42790	0.28708	4.86	4.11
S3	0-300	Suppr	40.6	1.92624	0.35552	0.41967	0.25228	4.98	5.14
S3	0-300	Suppr	66.3	2.52009	0.60617	0.55133	0.23923	5.32	4.38
S3	0-300	Suppr	73.6	3.48820	0.87217	1.67044	0.24793	6.02	5.36
S3	0-300	Suppr	19.3	2.84944	0.49363	0.75705	0.34798	5.38	4.50
S3	0-300	Suppr	26.1	2.74964	0.51665	1.86793	0.34363	5.77	4.88
S3	0-300	Suppr	88.6	4.07206	0.72126	0.85579	0.25663	6.15	5.28
S3	0-300	Suppr	14.3	3.10894	0.38365	0.81465	0.24358	5.54	4.65
S3	0-300	Suppr	45.6	4.47128	0.52944	1.06151	0.24793	6.02	5.39
C3	0-300	Cond	11.7	4.16188	0.56780	1.60461	0.46107	6.11	5.57
C3	0-300	Cond	24.5	3.46325	0.30181	1.31660	0.45672	6.11	5.26
C3	0-300	Cond	15.4	1.94121	1.33766	0.60893	0.44802	5.96	4.77
C3	0-300	Cond	36.9	3.93732	0.28134	1.12734	0.43062	6.00	5.20
C3	0-300	Cond	26.8	5.31464	1.46555	2.22177	0.47412	7.12	6.47
C3	0-300	Cond	24.8	3.74769	0.58571	1.60461	0.50892	6.75	6.19
C3	0-300	Cond	73.6	3.85249	0.73661	1.25900	0.51327	6.23	5.51
C3	0-300	Cond	17.8	3.19876	0.51409	0.76527	0.46542	5.99	4.94
C3	0-300	Cond	5.5	2.98418	1.18165	0.81465	0.46977	6.54	5.34
C3	0-300	Cond	24.8	3.74769	0.58571	1.60461	0.50892	6.52	5.70

Raw data of the analysis of suppressive and conducive soils from three fields in Kiepersol, South Africa

Sample	Depth (mm)	Suppr/Cond	P ($mg\ kg^{-1}$)	Ca ($cmol_c\ kg^{-1}$)	K ($cmol_c\ kg^{-1}$)	Mg ($cmol_c\ kg^{-1}$)	Na ($cmol_c\ kg^{-1}$)	pH(H_2O)	pH(KCl)
S3	300-600	Suppr	4.2	0.79345	0.06394	0.32915	0.28708	4.83	4.14
S3	300-600	Suppr	4.3	0.66371	0.09463	0.16458	0.23923	4.68	4.07
S3	300-600	Suppr	6.3	0.97809	0.14067	0.59247	0.23054	5.09	4.17
S3	300-600	Suppr	4.8	1.80149	0.14323	0.51841	0.25663	5.47	4.56
S3	300-600	Suppr	4.8	1.55197	0.12533	0.32092	0.23054	5.08	4.25
C3	300-600	Cond	2.5	0.46909	0.07673	0.18103	0.45672	4.58	4.10
C3	300-600	Cond	2.9	3.03408	0.76219	1.39066	0.46107	7.11	6.55
C3	300-600	Cond	1.1	2.59993	0.51921	1.25900	0.47847	6.61	6.02
C3	300-600	Cond	1.2	0.50402	0.21996	0.20572	0.41757	5.22	4.17
C3	300-600	Cond	1.1	2.59993	0.51921	1.25900	0.47847	6.43	5.67
S3	600-900	Suppr	4.4	0.80343	0.08185	0.45258	0.21749	5.04	4.36
S3	600-900	Suppr	2.6	0.64374	0.11254	0.31269	0.30013	4.93	4.21
S3	600-900	Suppr	2.8	0.73357	0.14579	0.41144	0.23488	4.90	4.24
S3	600-900	Suppr	2.9	0.71361	0.10486	0.21395	0.23488	5.14	4.22
S3	600-900	Suppr	2.9	0.49404	0.09719	0.10697	0.23488	4.55	4.11
C3	600-900	Cond	1	0.43415	0.02046	0.14812	0.46107	4.33	4.10
C3	600-900	Cond	1	1.95120	0.26856	0.99568	0.48282	6.89	6.51
C3	600-900	Cond	1.1	1.00803	0.23531	0.57601	0.49587	5.78	4.81
C3	600-900	Cond	0.9	0.39423	0.14835	0.13166	0.43932	4.75	4.09
C3	600-900	Cond	1.1	1.00803	0.23531	0.57601	0.49587	5.73	4.78

Raw data of the analysis of suppressive and conducive soils from three fields in Kiepersol, South Africa

Sample	Depth (mm)	Suppr/Cond	NH_4 ($cmol_c\ kg^{-1}$)	NO_3 ($cmol_c\ kg^{-1}$)	Cu ($mg\ kg^{-1}$)	Fe ($mg\ kg^{-1}$)	Mn ($mg\ kg^{-1}$)	Zn ($mg\ kg^{-1}$)
S3	0-300	Suppr	0.06519	0.06069	5.04	89.96	22.73	4.68
S3	0-300	Suppr	0.10741	0.07261	3.20	118.08	15.08	4.10
S3	0-300	Suppr	0.11486	0.03685	5.76	91.31	12.38	5.00
S3	0-300	Suppr	0.19165	0.07021	3.83	81.18	13.95	5.36
S3	0-300	Suppr	0.08506	0.06828	3.83	79.61	19.58	6.89
S3	0-300	Suppr	0.10121	0.06720	2.25	66.11	11.48	5.54
S3	0-300	Suppr	0.11859	0.04660	6.80	85.23	14.18	6.84
S3	0-300	Suppr	0.04574	0.02174	2.61	77.81	18.68	4.19
S3	0-300	Suppr	0.13577	0.13704	2.07	67.01	12.15	4.05
S3	0-300	Suppr	0.00331	0.03348	3.47	90.86	38.03	3.69
C3	0-300	Cond	0.10473	0.06268	2.88	59.85	34.65	3.87
C3	0-300	Cond	0.06437	0.04853	1.94	44.42	18.45	6.98
C3	0-300	Cond	0.15853	0.03679	2.43	46.26	5.18	2.25
C3	0-300	Cond	0.03125	0.02294	1.22	25.16	5.40	1.26
C3	0-300	Cond	0.07161	0.06930	0.81	15.53	2.25	1.04
C3	0-300	Cond	0.03332	0.03558	0.63	16.92	1.17	0.81
C3	0-300	Cond	0.09231	0.04010	2.57	36.18	6.35	1.89
C3	0-300	Cond	0.04574	0.02174	0.68	16.56	0.72	0.81
C3	0-300	Cond	0.08382	0.03757	0.50	11.43	0.95	0.72
C3	0-300	Cond	0.03332	0.03558	0.63	16.92	1.17	0.81

Raw data of the analysis of suppressive and conducive soils from three fields in Kiepersol, South Africa

Sample	Depth (mm)	Suppr/Cond	NH_4 ($cmol_c\ kg^{-1}$)	NO_3 ($cmol_c\ kg^{-1}$)	Cu ($mg\ kg^{-1}$)	Fe ($mg\ kg^{-1}$)	Mn ($mg\ kg^{-1}$)	Zn ($mg\ kg^{-1}$)
S3	300-600	Suppr	0.21297	0.08273	0.81	26.51	0.90	0.86
S3	300-600	Suppr	0.11714	0.04582	0.95	33.71	0.77	0.59
S3	300-600	Suppr	0.12542	0.03685	1.22	38.21	1.08	0.54
S3	300-600	Suppr	0.06126	0.03378	1.62	32.81	1.17	1.13
S3	300-600	Suppr	0.14343	0.03324	0.00	32.13	1.76	1.40
C3	300-600	Cond	0.08403	0.04462	1.04	19.17	0.54	0.68
C3	300-600	Cond	0.10617	0.05527	1.89	46.62	21.60	1.85
C3	300-600	Cond	0.12107	0.03396	4.91	49.10	17.33	5.58
C3	300-600	Cond	0.12232	0.05853	0.36	40.20	20.57	3.21
C3	300-600	Cond	0.12107	0.03396	4.91	49.10	17.33	5.58
S3	600-900	Suppr	0.07389	0.05419	0.54	26.06	1.94	1.04
S3	600-900	Suppr	0.12604	0.05925	0.81	23.13	0.81	0.54
S3	600-900	Suppr	0.06519	0.04444	0.54	25.38	0.72	0.63
S3	600-900	Suppr	0.11362	0.04263	0.90	22.23	0.59	0.50
S3	600-900	Suppr	0.07140	0.03035	0.00	24.71	0.63	0.72
C3	600-900	Cond	0.09872	0.04696	2.61	71.33	41.85	3.20
C3	600-900	Cond	0.12604	0.08092	2.12	24.93	4.64	1.40
C3	600-900	Cond	0.08879	0.02637	1.89	47.30	15.53	2.75
C3	600-900	Cond	0.06023	0.05202	0.72	31.98	30.30	1.43
C3	600-900	Cond	0.08879	0.02637	1.89	47.30	15.53	2.75

APPENDIX I.

Mean soil parameter with the standard deviation and t-test probability at a 5% level of significance analysed at different sampling depths in suppressive and conducive soils of three sampling fields in Kiepersol, South Africa

Parameter	Unit	Soil Depth	Field	Suppressive/ Conducive	Sample Size	Mean	Standard Deviation	t-test probability
P	mg kg^{-1}	0-300 mm	1	Suppressive	10	12.54	5.58	0.038
				Conducive	10	22.91	12.91	
			2	Suppressive	10	75.12	22.60	0.004
				Conducive	10	42.60	22.00	
			3	Suppressive	10	50.52	27.02	0.031
				Conducive	10	26.18	18.84	
P	mg kg^{-1}	300-600 mm	1	Suppressive	5	1.280	0.4764	0.005
				Conducive	5	2.900	0.8155	
			2	Suppressive	5	6.520	1.551	< 0.001
				Conducive	5	2.540	0.8142	
			3	Suppressive	5	4.880	0.8408	< 0.001
				Conducive	5	1.760	0.8706	
P	mg kg^{-1}	600-900 mm	1	Suppressive	5	1.800	1.463	0.341
				Conducive	5	3.480	3.413	
			2	Suppressive	5	3.720	0.6611	0.657
				Conducive	5	1.720	4.639	
			3	Suppressive	5	3.120	0.7259	0.003
				Conducive	5	1.020	0.08367	

Mean soil parameter with the standard deviation and t-test probability at a 5% level of significance analysed at different sampling depths in suppressive and conducive soils of three sampling fields in Kiepersol, South Africa

Parameter	Unit	Soil Depth	Field	Suppressive/ Conducive	Sample Size	Mean	Standard Deviation	t-test probability
Ca	$cmol_c\ kg^{-1}$	0-300 mm	1	Suppressive	10	3.333	0.6859	0.920
				Conducive	10	3.364	0.6759	
			2	Suppressive	10	3.307	2.112	0.015
				Conducive	10	1.252	0.8438	
			3	Suppressive	10	3.262	0.9088	0.360
				Conducive	10	3.635	0.8676	
Ca	$cmol_c\ kg^{-1}$	300-600 mm	1	Suppressive	5	1.968	0.3429	0.034
				Conducive	5	1.535	0.1646	
			2	Suppressive	5	0.9322	0.6444	0.247
				Conducive	5	0.5360	0.1837	
			3	Suppressive	5	1.158	0.4947	0.288
				Conducive	5	1.841	1.250	
Ca	$cmol_c\ kg^{-1}$	600-900 mm	1	Suppressive	5	1.709	0.3159	0.092
				Conducive	5	1.394	0.1873	
			2	Suppressive	5	0.4930	0.1432	0.806
				Conducive	5	0.5380	0.3682	
			3	Suppressive	5	0.6777	0.1174	0.378
				Conducive	5	0.9591	0.6292	

Mean soil parameter with the standard deviation and t-test probability at a 5% level of significance analysed at different sampling depths in suppressive and conducive soils of three sampling fields in Kiepersol, South Africa

Parameter	Unit	Soil Depth	Field	Suppressive/ Conducive	Sample Size	Mean	Standard Deviation	t-test probability
K	$cmol_c\ kg^{-1}$	0-300 mm	1	Suppressive	10	0.4399	0.2140	0.384
				Conducive	10	0.5174	0.1725	
			2	Suppressive	10	0.3987	0.1900	0.029
				Conducive	10	0.2417	0.03483	
			3	Suppressive	10	0.5484	0.1657	0.175
				Conducive	10	0.7558	0.4225	
K	$cmol_c\ kg^{-1}$	300-600 mm	1	Suppressive	5	0.2353	0.1444	0.179
				Conducive	5	0.3775	0.1606	
			2	Suppressive	5	0.1356	0.02652	0.200
				Conducive	5	0.1550	0.01634	
			3	Suppressive	5	0.1136	0.03382	0.065
				Conducive	5	0.4195	0.2714	
K	$cmol_c\ kg^{-1}$	600-900 mm	1	Suppressive	5	0.2573	0.1255	0.289
				Conducive	5	0.3688	0.1800	
			2	Suppressive	5	0.09924	0.01135	0.012
				Conducive	5	0.1396	0.02536	
			3	Suppressive	5	0.1084	0.02376	0.181
				Conducive	5	0.1816	0.1005	

Mean soil parameter with the standard deviation and t-test probability at a 5% level of significance analysed at different sampling depths in suppressive and conducive soils of three sampling fields in Kiepersol, South Africa

Parameter	Unit	Soil Depth	Field	Suppressive/ Conducive	Sample Size	Mean	Standard Deviation	t-test probability
Mg	$cmol_c\ kg^{-1}$	0-300 mm	1	Suppressive	10	1.045	0.1399	0.684
				Conducive	10	1.087	0.2866	
			2	Suppressive	10	1.006	0.4485	0.010
				Conducive	10	0.4534	0.4087	
			3	Suppressive	10	0.9800	0.5080	0.178
				Conducive	10	1.293	0.4897	
Mg	$cmol_c\ kg^{-1}$	300-600 mm	1	Suppressive	5	0.8344	0.09284	0.127
				Conducive	5	0.7274	0.1055	
			2	Suppressive	5	0.3785	0.1902	0.276
				Conducive	5	0.2534	0.1452	
			3	Suppressive	5	0.3851	0.1708	0.160
				Conducive	5	0.8591	0.6101	
Mg	$cmol_c\ kg^{-1}$	600-900 mm	1	Suppressive	5	1.019	0.2861	0.070
				Conducive	5	0.7373	0.09501	
			2	Suppressive	5	0.2715	0.06685	0.040
				Conducive	5	0.1580	0.07923	
			3	Suppressive	5	0.2995	0.1419	0.313
				Conducive	5	0.4855	0.3591	

Mean soil parameter with the standard deviation and t-test probability at a 5% level of significance analysed at different sampling depths in suppressive and conducive soils of three sampling fields in Kiepersol, South Africa

Parameter	Unit	Soil Depth	Field	Suppressive/ Conducive	Sample Size	Mean	Standard Deviation	t-test probability
Na	$cmol_c\ kg^{-1}$	0-300 mm	1	Suppressive	10	0.1501	0.1715	0.364
				Conducive	10	0.2023	0.02173	
			2	Suppressive	10	0.2397	0.09935	< 0.001
				Conducive	10	0.008917	0.01060	
			3	Suppressive	10	0.2697	0.04277	< 0.001
				Conducive	10	0.4737	0.02808	
Na	$cmol_c\ kg^{-1}$	300-600 mm	1	Suppressive	5	0.1000	0.1505	0.224
				Conducive	5	0.1983	0.04640	
			2	Suppressive	5	0.1983	0.09194	0.009
				Conducive	5	0.0008700	0.001945	
			3	Suppressive	5	0.2488	0.02390	< 0.001
				Conducive	5	0.4585	0.02491	
Na	$cmol_c\ kg^{-1}$	600-900 mm	1	Suppressive	5	0.1014	0.1661	0.190
				Conducive	5	0.2192	0.03799	
			2	Suppressive	5	0.2001	0.1140	0.019
				Conducive	5	0.008698	0.01259	
			3	Suppressive	5	0.2445	0.03202	< 0.001
				Conducive	5	0.4750	0.02449	

Mean soil parameter with the standard deviation and t-test probability at a 5% level of significance analysed at different sampling depths in suppressive and conducive soils of three sampling fields in Kiepersol, South Africa

Parameter	Soil Depth	Field	Suppressive/ Conducive	Sample Size	Mean	Standard Deviation	t-test probability
$pH(H_2O)$	0-300 mm	1	Suppressive	10	6.307	0.4078	0.040
			Conducive	10	6.625	0.1452	
		2	Suppressive	10	5.501	0.5935	0.012
			Conducive	10	4.901	0.2541	
		3	Suppressive	10	5.602	0.4584	0.001
			Conducive	10	6.333	0.3871	
$pH(H_2O)$	300-600 mm	1	Suppressive	5	5.914	0.4097	0.802
			Conducive	5	5.856	0.2855	
		2	Suppressive	5	5.104	0.3385	0.123
			Conducive	5	4.808	0.07918	
		3	Suppressive	5	5.030	0.3009	0.111
			Conducive	5	5.990	1.050	
$pH(H_2O)$	600-900 mm	1	Suppressive	5	5.881	0.3321	0.856
			Conducive	5	5.918	0.2701	
		2	Suppressive	5	4.774	0.07021	0.480
			Conducive	5	4.816	0.1057	
		3	Suppressive	5	4.912	0.2235	0.265
			Conducive	5	5.496	0.9993	

Mean soil parameter with the standard deviation and t-test probability at a 5% level of significance analysed at different sampling depths in suppressive and conducive soils of three sampling fields in Kiepersol, South Africa

Parameter	Soil Depth	Field	Suppressive/ Conducive	Sample Size	Mean	Standard Deviation	t-test probability
pH(KCl)	0-300 mm	1	Suppressive	10	5.449	0.4082	0.033
			Conducive	10	5.784	0.2118	
		2	Suppressive	10	4.730	0.4856	0.002
			Conducive	10	4.061	0.2213	
		3	Suppressive	10	4.908	0.4726	0.017
			Conducive	10	5.495	0.5251	
pH(KCl)	300-600 mm	1	Suppressive	5	5.230	0.4510	0.767
			Conducive	5	5.302	0.2688	
		2	Suppressive	5	4.228	0.1988	0.154
			Conducive	5	4.072	0.02280	
		3	Suppressive	5	4.238	0.1912	0.099
			Conducive	5	5.302	1.111	
pH(KCl)	600-900 mm	1	Suppressive	5	5.290	0.3530	0.431
			Conducive	5	5.446	0.2281	
		2	Suppressive	5	4.146	0.04037	0.767
			Conducive	5	4.158	0.07759	
		3	Suppressive	5	4.228	0.08927	0.227
			Conducive	5	4.858	0.9877	

Mean soil parameter with the standard deviation and t-test probability at a 5% level of significance analysed at different sampling depths in suppressive and conducive soils of three sampling fields in Kiepersol, South Africa

Parameter	Unit	Soil Depth	Field	Suppressive/ Conducive	Sample Size	Mean	Standard Deviation	t-test probability
NH_4^+	$cmol_c\ kg^{-1}$	0-300 mm	1	Suppressive	10	0.08554	0.04063	0.111
				Conducive	10	0.1277	0.06832	
			2	Suppressive	10	0.1395	0.1150	0.175
				Conducive	10	0.08421	0.03556	
			3	Suppressive	10	0.09688	0.05157	0.242
				Conducive	10	0.07190	0.04015	
NH_4^+	$cmol_c\ kg^{-1}$	300-600 mm	1	Suppressive	5	0.1320	0.07352	0.388
				Conducive	5	0.09971	0.02925	
			2	Suppressive	5	0.05940	0.02327	0.270
				Conducive	5	0.1028	0.07415	
			3	Suppressive	5	0.1320	0.05467	0.448
				Conducive	5	0.1109	0.01644	
NH_4^+	$cmol_c\ kg^{-1}$	600-900 mm	1	Suppressive	5	0.1222	0.06780	0.710
				Conducive	5	0.1077	0.04973	
			2	Suppressive	5	0.1046	0.03910	0.231
				Conducive	5	0.07757	0.02548	
			3	Suppressive	5	0.09003	0.02774	0.883
				Conducive	5	0.09251	0.02362	

Mean soil parameter with the standard deviation and t-test probability at a 5% level of significance analysed at different sampling depths in suppressive and conducive soils of three sampling fields in Kiepersol, South Africa

Parameter	Unit	Soil Depth	Field	Suppressive/ Conducive	Sample Size	Mean	Standard Deviation	t-test probability
NO_3^-	$cmol_c\ kg^{-1}$	0-300 mm	1	Suppressive	10	0.05678	0.01899	0.489
				Conducive	10	0.06279	0.01904	
			2	Suppressive	10	0.06635	0.02604	0.099
				Conducive	10	0.04768	0.02172	
			3	Suppressive	10	0.06147	0.03194	0.092
				Conducive	10	0.04108	0.01533	
NO_3^-	$cmol_c\ kg^{-1}$	300-600 mm	1	Suppressive	5	0.06438	0.03856	0.541
				Conducive	5	0.08183	0.04733	
			2	Suppressive	5	0.03799	0.02095	0.770
				Conducive	5	0.04168	0.01745	
			3	Suppressive	5	0.04648	0.02088	0.912
				Conducive	5	0.04527	0.01153	
NO_3^-	$cmol_c\ kg^{-1}$	600-900 mm	1	Suppressive	5	0.07673	0.03106	0.761
				Conducive	5	0.07189	0.01458	
			2	Suppressive	5	0.06948	0.01569	0.002
				Conducive	5	0.03351	0.008830	
			3	Suppressive	5	0.04617	0.01119	0.976
				Conducive	5	0.04653	0.02250	

Mean soil parameter with the standard deviation and t-test probability at a 5% level of significance analysed at different sampling depths in suppressive and conducive soils of three sampling fields in Kiepersol, South Africa

Parameter	Unit	Soil Depth	Field	Suppressive/ Conducive	Sample Size	Mean	Standard Deviation	t-test probability
Cu	mg kg^{-1}	0-300 mm	1	Suppressive	10	2.985	0.5646	0.971
				Conducive	10	2.973	0.8504	
			2	Suppressive	10	2.784	0.8634	0.166
				Conducive	10	2.346	0.3713	
			3	Suppressive	10	3.886	1.549	< 0.001
				Conducive	10	1.429	0.9308	
Cu	mg kg^{-1}	300-600 mm	1	Suppressive	5	2.028	0.5569	0.918
				Conducive	5	2.000	0.1319	
			2	Suppressive	5	1.750	0.3669	0.162
				Conducive	5	1.450	0.2354	
			3	Suppressive	4	1.150	0.3566	0.204
				Conducive	5	2.622	2.158	
Cu	mg kg^{-1}	600-900 mm	1	Suppressive	5	2.296	0.7341	0.587
				Conducive	5	2.512	0.4346	
			2	Suppressive	5	1.424	0.5700	0.582
				Conducive	5	1.224	0.5309	
			3	Suppressive	4	0.6975	0.1855	0.016
				Conducive	5	1.846	0.6947	

Mean soil parameter with the standard deviation and t-test probability at a 5% level of significance analysed at different sampling depths in suppressive and conducive soils of three sampling fields in Kiepersol, South Africa

Parameter	Unit	Soil Depth	Field	Suppressive/ Conducive	Sample Size	Mean	Standard Deviation	t-test probability
Fe	mg kg^{-1}	0-300 mm	1	Suppressive	10	141.8	22.38	0.683
				Conducive	10	134.6	48.94	
			2	Suppressive	10	99.03	20.07	0.009
				Conducive	10	77.95	6.637	
			3	Suppressive	10	84.72	14.79	< 0.001
				Conducive	10	28.92	16.63	
Fe	mg kg^{-1}	300-600 mm	1	Suppressive	5	32.11	6.101	0.618
				Conducive	5	30.49	3.339	
			2	Suppressive	5	49.10	10.89	0.017
				Conducive	5	30.57	3.038	
			3	Suppressive	5	32.67	4.184	0.208
				Conducive	5	40.84	13.65	
Fe	mg kg^{-1}	600-900 mm	1	Suppressive	5	28.76	8.883	0.415
				Conducive	5	25.16	2.931	
			2	Suppressive	5	30.46	7.895	0.232
				Conducive	5	23.70	9.631	
			3	Suppressive	5	24.30	1.588	0.064
				Conducive	5	44.57	17.86	

Mean soil parameter with the standard deviation and t-test probability at a 5% level of significance analysed at different sampling depths in suppressive and conducive soils of three sampling fields in Kiepersol, South Africa

Parameter	Unit	Soil Depth	Field	Suppressive/ Conducive	Sample Size	Mean	Standard Deviation	t-test probability
Mn	mg kg^{-1}	0-300 mm	1	Suppressive	10	41.04	13.54	0.684
				Conducive	10	38.75	11.07	
			2	Suppressive	10	34.98	19.42	0.031
				Conducive	10	19.00	6.274	
			3	Suppressive	10	17.82	7.987	0.028
				Conducive	10	7.629	10.88	
Mn	mg kg^{-1}	300-600 mm	1	Suppressive	5	6.546	6.305	0.622
				Conducive	5	8.796	7.523	
			2	Suppressive	5	2.064	0.7410	0.485
				Conducive	5	2.714	1.844	
			3	Suppressive	5	1.136	0.3819	0.020
				Conducive	5	15.47	8.565	
Mn	mg kg^{-1}	600-900 mm	1	Suppressive	5	7.626	5.749	0.553
				Conducive	5	11.66	13.41	
			2	Suppressive	5	1.558	0.5531	0.222
				Conducive	5	6.170	7.127	
			3	Suppressive	5	0.9380	0.5665	0.034
				Conducive	5	21.57	14.55	

Mean soil parameter with the standard deviation and t-test probability at a 5% level of significance analysed at different sampling depths in suppressive and conducive soils of three sampling fields in Kiepersol, South Africa

Parameter	Unit	Soil Depth	Field	Suppressive/ Conducive	Sample Size	Mean	Standard Deviation	t-test probability
Zn	mg kg^{-1}	0-300 mm	1	Suppressive	10	2.806	0.5958	0.412
				Conducive	10	2.465	1.139	
			2	Suppressive	10	5.398	1.842	0.001
				Conducive	10	2.711	1.261	
			3	Suppressive	10	5.034	1.133	< 0.001
				Conducive	10	2.044	1.992	
Zn	mg kg^{-1}	300-600 mm	1	Suppressive	5	0.8580	0.2711	0.009
				Conducive	5	0.3640	0.1691	
			2	Suppressive	5	0.5240	0.3454	0.821
				Conducive	5	0.4820	0.2052	
			3	Suppressive	5	0.9040	0.3640	0.065
				Conducive	5	3.380	2.199	
Zn	mg kg^{-1}	600-900 mm	1	Suppressive	5	1.216	0.8115	0.166
				Conducive	5	0.5600	0.5169	
			2	Suppressive	5	0.704	0.5917	0.701
				Conducive	5	0.5940	0.08050	
			3	Suppressive	5	0.6860	0.2154	0.010
				Conducive	5	2.306	0.8339	