

**Effect of soil factors on parasitic nematodes of sugarcane in KwaZulu-
Natal, South Africa.**

by

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ABSTRACT

Nematicides are not only expensive and unaffordable to small-scale farmers but are also harmful to the environment as they kill both the target organisms and non-target micro and macro-organisms, thereby destabilising the ecosystem. Most developed countries have or are in the process of banning use of chemicals for pest management, implying that agricultural products from developing countries using pesticides will not be marketed in the developed countries. In sugarcane fields, plant parasitic nematodes pose a serious problem and their attacks on sett roots during the germination period lead to fewer buds germinating as most buds abort and germination may be delayed in others. Those that germinate later are then faced with competition for food, space and light from the “older” shoots and often die. The attacks on shoot roots may lead to inefficient uptake of water and nutrients by the plant thereby leading to stunted plants. Fewer and shorter sugarcane stalks due to nematode attacks result in poor yield.

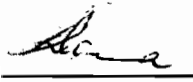
Agricultural systems based on monoculture are rarely successful in the long term and because sugarcane fields have been monocultured for a very long time, they are losing their productive capacity and this is termed “yield decline”. Instead of using nematicides, alternative control methods can be used for the management of nematode communities. Research has shown on other plants that nematode communities dominated by *Helicotylenchus dihystra* are less pathogenic to the plants than other ectoparasitic nematodes, e.g. *Xiphinema* spp. and *Paratrichodorus* spp. A study conducted as a pot experiment showed that sugarcane grown in soil with high *H. dihystra* grew taller and produced greater root and aerial biomass than one grown in *Xiphinema elongatum* infested soil. To induce a nematode community dominated by *H. dihystra* in the field, two strategies were followed: (i) abiotic factors that influence the nematode’s environment were identified, (ii) organic amendments and inter-cropping were used to improve the sugarcane growth, modify the environment and decrease competition among species within a community. Application of organic matter to the soil improves soil properties such as water infiltration, water holding capacity, erodibility and nutrient cycling, increases suppressiveness of soils to plant parasitic nematode and stimulates other anti-nematode micro-organisms, e.g. nematode-trapping fungi. Organic amendments were therefore used in this study not only as screens to protect sugarcane roots from nematode attacks but also to manipulate nematode communities for the less pathogenic species, *H. dihystra*. In a field study where organic amendments were used, plots treated with filter cake, thume + filter cake, trash + filter cake and Temik (aldicarb) had high percentages of *H. dihystra* while control plots had high percentages of *X. elongatum*. Plots with high *H. dihystra* counts produced more yield than plots heavily infested with *X. elongatum*. The distribution of nematode species during a sugarcane crop cycle (time) and across the field (space) was found to be stable. Certain soil types and elements found in soil and sugarcane leaves were found to be correlated to certain species, e.g. *H. dihystra* was negatively correlated to soil sulphur, medium and coarse sand while *X. elongatum* was positively correlated to these soil types and elements found in the soil. Sugarcane leaves with high levels of Ca, Zn, Cu and Fe were found in areas

with high percentages of *H. dihystra* while the reverse was true for *X. elongatum*. Organic amendments can be used by sugarcane small-scale farmers in manipulating the nematode communities for the less pathogenic species, *H. dihystra* and to improve yield as organic matter is full of nutrients.

PREFACE

The experimental work described in this dissertation was carried out at the South African Sugar Association Experiment Station, Mt. Edgecombe, in collaboration with the University of Natal, Durban and the Institut de recherche pour le développement, France, from October 2000 to September 2003, under the supervision of Drs. P. Cadet, V.W. Spaul and Prof. C.C. Appleton.

This study represents original work by the author and has not otherwise been submitted in any form for any degree or diploma to any tertiary institution. Where use has been made of the work of others, it is duly acknowledged in the text.



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This work is dedicated to my whole family,
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CHAPTER 1

INTRODUCTION

Plant-parasitic nematodes are the unseen enemy of plants that inhabit the soil environment (Ferraz & Brown, 2002). They cause some of the most intractable problems encountered in agricultural economy. They inhibit root growth and hence overall plant development and this results in poor crop performance and crop failure (Kleynhans et al., 1996). Five to ten percent of agricultural production is lost to nematodes in developed countries and the number is greater in less developed countries (Viglierchio, 1991). The damage caused by plant parasitic nematodes goes unnoticed or is usually attributed to other growth-limiting factors (Kleynhans et al., 1996; Ferraz & Brown, 2002). This is because many growers are unaware of nematodes and the potential they have on limiting plant growth.

Plant-parasitic nematodes are found in all cultivated land (Page & Bridge, 1993). They spend part or all of their lives in the soil and are to some extent affected by edaphic and biotic factors (Stirling, 1991; Villenave & Duponnois, 1998). Abiotic factors may be influential in soils where the survival and population increases are favoured compared to seemingly homogenous soils with smaller increases in nematode density (Alby et al., 1983; Noe & Barker, 1985). This may explain why nematodes typically have a patchy distribution in the field (Ferris et al., 1990). The distribution of nematodes in a sugarcane field was determined and the possibility that abiotic soil factors were responsible was looked at.

Plant-parasitic nematodes need a host plant in order to survive and host plants determine the structure of the nematode community associated with them (Norton, 1989; Niblack & Bernard, 1985; Freckman & Caswell, 1985; Cadet & Thioulouse, 1998; Villenave & Cadet, 1999). Plants are autotrophs and supply nematodes with the food needed to maintain their populations (Yeates, 1999). Any factor affecting the host plant, such as increases in microbial populations, also affects the nematodes (Freckman & Caswell, 1985). All plants are attacked by one or more species of phyto-nematode species (Barker et al., 1994). Some nematodes are host-specific, e.g. potatoes (*Solanum tuberosum*) are hosts of a potato cyst nematode *Globodera rostochiensis*, while sugarbeet (*Beta vulgaris*) is a host to a beet cyst nematode *Heterodera schachtii*, (Yeates & Bongers, 1999).

The relationship between plants and nematodes is to some extent influenced by environmental factors (Francel, 1993; Griffin, et al., 1996; Barker & Koenning, 1998) and the environment, together with the host plant, is important in determining the structure of a plant-parasitic nematode community (Villenave & Cadet, 1999). Nematode populations may be affected directly by edaphic factors or indirectly through the response of the host to its environment (Francel, 1993). Some of the environmental factors that may limit nematode populations are pH, by indirectly or directly affecting their populations through their food source,

the host plant (Yeates, 1987); soil texture, by restricting movement of nematodes towards roots and mates (Villenave et al., 1997) and temperature and moisture, during the reproductive stage (Norton, 1989). The soil environment, through physical and physiological impact on nematodes and also through favourable and adverse effects on the host plant, may directly or indirectly affect the growth of nematode populations respectively.

The extent to which roots are damaged by nematodes not only depends on nematode numbers but also on the balance between the species within the community (Villenave & Cadet, 1998). Some nematodes are more pathogenic while others are less pathogenic. Nematode communities dominated by *X. elongatum* caused more damage to millet than communities dominated by *H. dihystra*, which is considered to be a weak pathogen. Circumstantial evidence from West Africa suggests that the same may be happening in sugarcane (Cadet et al., 2001). In studies conducted in Burkina Faso and South Africa on ratoon cane of the same variety and grown on similar sandy soil, sugarcane stalks grew taller in Burkina Faso than in South Africa as the dominant ectoparasite was *H. dihystra* in Burkina Faso while *X. elongatum* was dominant in South Africa (Spaull & Cadet, 1991). The damage nematodes cause to the roots reduces the roots' efficiency in water and nutrient uptake (Spaull & Cadet, 2001).

In the South African sugar industry, plant-parasitic nematodes limit sugarcane yield (Cadet et al., 2000). These nematodes can be controlled by both chemical and non-chemical methods. Carbamate and organophosphate nematicides are currently used to reduce nematode numbers early in the season when crops are most vulnerable to nematode damage, thereby reducing crop loss (Cadet et al., 2000; Stirling & Blair, 2000). A nematicide trial on plant cane in sandy soils in South Africa and Burkina Faso showed increases in the length and number of stalks respectively (Cadet & Spaull, 1985). In both localities, the sugarcane variety was the same and the nematode communities were similar such that the attack on sett roots by *Meloidogyne* and *Pratylenchus* delayed the development of primary shoots. In South Africa, the dominant ectoparasites were *Xiphinema* spp. and *Paratrichodorus* spp. and their attack on shoot roots restricted water uptake and thus limited stalk elongation (Cadet & Spaull, 1985).

The problem is that these chemicals kill both the target pests and other non-target micro and macroorganisms, thereby destabilising the ecosystem and also have deleterious effects on human health and environment (Oka & Yermiyahu, 2002). Nematicide use has also resulted in contamination of groundwater with adverse effects on animal and human health (Bauske et al., 1994). Most developed countries have or are in the process of banning the use of chemicals for pest management, which means that agricultural products from developing countries still using pesticides will not be accepted and marketed in developed countries. A number of non-chemical alternatives to nematicides are available for management of plant-parasitic nematodes e.g. crop rotation, trap cropping, fallowing, plant resistance and organic amendments (McSorley & Duncan, 1995; Kleynhans, 1996). However, some of these methods are not very effective

when used alone and need to be integrated with other methods in order to achieve optimal nematode management. Organic amendments have been shown to be successful in nematode control (Rodríguez-Kábana, 1986) as they contain nematotoxic chemicals that are released into the soil and suppress nematode populations, e.g. neem, *Azadirachta indica*, marigolds, *Tagetes* spp. and castor bean, *Ricinus communis* (Stirling, 1991), sunn hemp, *Crotalaria* spp., sesame, *Sesamum indicum* and velvet bean, *Mucuna* spp. (Ferraz & Brown, 2002). The practice of incorporating crop residues and animal manures into soil as soil amendments is as old as agriculture itself and when they decompose due to microbial activity they produce chemicals that can be detrimental to nematodes.

A change in the structure of the nematode community may bring about a change in the way they parasitise the host plants. Instead of using nematicides to control nematodes, Spaul & Cadet (2001) propose that one way of controlling nematodes is to manipulate their populations in favour of the less pathogenic species. This may only be possible if factors affecting the equilibrium between nematode species in the community are known. The environment could affect the nematodes' perception of root exudates and hence their ability to locate food and suitable mates.

The aim of this study therefore, was to determine whether organic amendments protect sugarcane roots from plant parasitic nematodes and succeed in manipulating nematode communities in favour of the less pathogenic species. If organic amendments can manage to do this, they can be used by small-scale farmers to increase their yield instead of using nematicides, which are not only expensive and unaffordable but also harmful to the environment.

Programme:

- i) To determine the relationship between nematode community structure and plant production.
- ii) To determine whether nematode communities change during the sugarcane growth period.
- iii) To determine whether nematode communities show spatial variation in the field.
- iv) To determine if abiotic soil factors are related with nematode distribution along the field.
- v) To determine if abiotic soil factors are related with nematode species balance?
- vi) To determine the spatial variability of leaf elements.
- vii) To determine if leaf elements are related with nematode species balance.
- viii) To determine if abiotic soil factors are related with leaf elements.
- ix) To manage nematode communities through the use of organic amendments.

CHAPTER 2

BIBLIOGRAPHY

2.1 BACKGROUND ON SUGARCANE

Sugarcane is a member of the family Gramineae (Soopramanien, 2000) and is a complex hybrid of *Saccharum officinarum*, the noble cane due to its thick and sweet stems, and other *Saccharum* spp. (Stirling et al., 2001). It is a tall, perennial grass with thick stems grown in more than 80 countries in the tropics and subtropics (Spaull & Cadet, 1990). It is an important crop in many tropical countries such as Mauritius, Jamaica, Swaziland and Dominican Republic as it is a major revenue source and its production has greatly increased in the past few decades (Hartemink & Wood, 1998). Four sugarcane species, *Saccharum officinarum*, *S. robustum*, *S. edule* and *S. spontaneum* are endemic to Papua New Guinea (Kuniata et al., 2001). Modern cultivars are hybrids of *S. officinarum*, *S. spontaneum*, *S. sinense* and *S. barberi* (Spaull & Cadet, 1990). During the period 1982 – 1987, the mean annual world sugarcane production exceeded 63 000 000 tons, with Brazil being the biggest producer. South Africa, together with Cuba, India, Brazil, Mexico, China, Thailand and Australia produced more than 60 % of the world total.

Sugarcane is propagated vegetatively (van Dillewijn, 1952) with the crop cycle beginning when setts with two or more nodes are planted (Spaull & Cadet, 1990). A few days after planting, thin and much branched sett roots emerge from the nodes (van Antwerpen, 1999) and support the initial growth of primary shoots developing from the axillary buds (Spaull & Cadet, 1990). Sett roots serve the plant for about two to three months until young shoots develop, producing their own shoot roots (Soopramanien, 2000). Shoot roots are thicker, succulent and less branched than sett roots. The root system of the plant is continually renewed, as new developing shoots grow their own roots (van Dillewijn, 1952). The young shoots grow into stalks with nodes, each of which has an axillary bud and a leaf. Inbetween the nodes are internodes where sucrose is stored. Sugarcane plants grow in stools made up of different number of stalks that mature when they are approximately 2–3 m long and 20–30 mm big (Spaull & Cadet, 1990). The leaves are made up of two parts, the leaf sheath and the leaf lamina (Soopramanien, 2000). The leaf sheath is the lower part of the leaf which wraps around the interode and its margins overlap at the base. The leaf lamina tapers towards the tip, has sharp margins, and has a white midrib on the upper surface. The crop is harvested between 12 and 24 months after planting depending on temperature and water availability.

Two economically important by-products of sugarcane are molasses, a liquid used to produce a range of alcohols, yeast and animal feed, and bagasse, a fibrous residue from cane stalks (Spaull & Cadet, 1990, Fernández & Pérez, 1996). Recently, it has been discovered that alcohol can be produced from any sugar-rich raw material like sugarcane (Mutton et al., 1996). Pieces of the sugar-containing raw material are

fermented so that the extraction of sugars and their fermentation occurs simultaneously. The end result of fermentation is then a mixture of yeast, liquor and bagasse. Once the alcohol component has been removed, the remainder can be processed to form livestock feed with high proteins, vitamins, carbohydrates, water and mineral salts. Bagasse has been used for industrial purposes for over 150 years (Fernández & Garcia, 1996). In Peru, it has been used to produce pulp and paper, filtering boards, pharmaceutical products, animal feed and other fibrous derivatives. In Mauritius, they have reduced dependency on fuel oil for electricity production by using bagasse instead (Kong Win Chang et al., 2001).

Development in the sugarcane industry is driven by the wish to convert the fuel value of trash (tops and leaves separated at harvest) and bagasse (Morris & Waldheim, 2001). Mason (2001) and Hassuani (2001) suggested that sugarcane trash (green and dried leaves) can provide an additional source of fibre to boost electricity export.

2.2 SOIL CHARACTERISTICS

2.2.1 Physical characteristics

The South African sugar industry uses a wide variety of soil types which differ in their properties (SASEX, 1999). The differences between these soil types can be attributed to the parent material from which they have formed, the environmental (topography, temperature, rainfall and drainage) and biological conditions under which they developed and are characterised by referring to their parent material and geographical locality. Dolerite has weathered to form heavy, deep red or shallow black clays, shales and Dwyka tillite have eroded to produce shallow, fine-grained soils while granite has produced coarse, abrasive, sandy loam soils. In South Africa, sugarcane is grown on about 400 000 ha of land with varying climatic and soil conditions (Meyer et al., 1996). Grey soils (Entisols) form about 60 % of the total area under cane followed by the red soils (Oxisols) which form 19 %, black Vertisols form 13 % and lastly brown humic Ultisols form 8 % (Meyer et al., 1996).

Most conditions are not suitable for maximum sugar production as soils are often shallow and on steep slopes (Platford & Bond, 1996). More than half the soils in the South African sugar industry are highly erodable and most cane is grown on steep slopes. High rainfall can lead to large amounts of soil being carried away in the runoff water. Burning cane before harvest can promote soil erosion but green cane harvesting and leaving cane tops scattered in the field can prevent soil erosion, conserve moisture and soil organic content, control weeds, increase compost and so benefit cane production (de Beer et al., 1996; Echavarria, 1996; Branauck, 1997; Viator, 2002).

Sugarcane production is harmful for soil in general, especially the physical aspect of soil (McGarry & Bristow, 2001). Conversion of virgin soils to arable cropping systems leads to the decline of organic matter (Johnston, 1986) due to reduced organic material inputs and an increased rate of organic matter decomposition (Haynes & Hamilton, 1999). Soil organic matter in soil decreases when soil is put under sugarcane as was observed in Australia (Wood, 1985), Papua New Guinea (Hartemink, 1998a) and South Africa (van Antwerpen & Meyer, 1996, Haynes et al., 2003). In Northern KwaZulu-Natal, loss of organic matter under sugarcane in both dryland and irrigated areas is common (van Antwerpen & Meyer, 1996).

Application of organic matter can reduce the density of compacted soils especially those with low organic matter (van Antwerpen & Meyer, 2001). The quantity and quality of soil organic matter influences physical properties related to soil moisture dynamics, erodibility and workability (Schjønning et al., 1994). According to Graham et al. (2002), green cane harvesting greatly improved the organic matter content and structural stability in inter-rows because they are covered by trash. It also improved soil physical properties in Thailand and in Fiji and led to increased sugarcane yields (Prammanee et al., 1996; Yang, 1996). Application of filter cake, a by-product from the sugar mill, raises soil organic matter levels, adds nutrients and raises the soil pH (Dee et al., 2002). Wynne & Meyer (2002) suggest that molasses can be used as a source of potassium to improve the soil structure and to increase biological activity of microorganisms such as fungi.

As noted above, the productive capacity of soil may be reduced by sugarcane monoculture as soils under monocultural systems generally contain lower concentrations and qualities of soil organic matter, less soil structural stability and reduced microbial activity (Moore et al., 2000). In South Africa, almost two-thirds of the soils are sands, sandy loams, loamy sands and sandy clay loams (Anon, 1999). Newly released sugarcane varieties have the potential to produce better yields but monoculture may already have resulted in a decline of soil quality thereby cancelling the positive contributions of the recently released cane varieties (Meyer & van Antwerpen, 2001). According to Henry (1995) sugarcane monocropping in Swaziland resulted in degradation of soil properties like surface crusting, low infiltration rate, high bulk density, and low organic matter, potassium and sodicity.

2.2.2 Chemical characteristics

In South Africa, soil acidification has been clearly demonstrated to occur under cane production (Haynes & Hamilton, 1999). A general decline in pH values is accompanied by a decrease in base cation concentrations and an increase in extractable aluminium on commercial fields on the south coast of KwaZulu-Natal (Schroeder, et al., 1994). A study conducted on the south coast and midlands of KwaZulu-Natal by Qongqo & van Antwerpen (2000) showed that pH, Ca and Mg decreased while Al increased as the period under sugarcane cultivation increased. In the sandy loam soil of the south coast, P was unchanged while K and organic matter decreased while in the Midlands clay soil P increased while K and organic

matter remained unchanged. In another study conducted in Glenrosa and Hutton in KwaZulu-Natal, the initial soil organic carbon content was 46 g/kg in both localities and under sugarcane monoculture it decreased to 34 g/kg and 13 g/kg respectively (Dominy et al., 2001). The higher organic matter content in Hutton was attributed to high clay content, which was 62 % in Hutton compared to 18 % in Glenrosa. As soil organic matter decreased, so did soil microbial biomass, basal respiration and aggregate stability.

Sugarcane waste products can change the chemical composition of soil upon decomposition. In India, incorporation of trash into sugarcane soil increased nitrogen and phosphorus availability to the plants (Jadhav, 1996). Stillage or vinasse, a by-product of ethanol production, has been used in Brazil and Australia as a source of potassium in sugarcane fields (Korndorfer & Anderson, 1997). It is also a good source of calcium and sulphur, and improves soil properties as it is rich in organic matter (Filho et al., 1996). Vinasse application increased sugarcane yield in São Paulo, Brazil. Drawbacks are salinity and the potentially harmful effects of adding large amounts of degradable sugars (Korndorfer & Anderson, 1997). Turner et al., (2002) also found that in Swaziland, potassium levels increased when vinasse was applied to sugarcane fields. A study conducted in sugarcane fields on termite mounds in KwaZulu-Natal showed that calcium, silicon and magnesium levels were higher inside the termite mounds than in other areas in the field (Cadet et al., 2002).

2.3 SOIL ORGANISMS ASSOCIATED WITH SUGARCANE

Deleterious soil organisms affecting the health and growth of sugarcane have increased under sugarcane monoculture and they include fungi of the species *Pachymetra chaunorhiza* and *Pythium arrhenomanes* (Magarey, 1996). On the other hand, beneficial soil organisms that may decrease in numbers because of monoculture include *Pseudomonas* spp. and fungi that may be naturally suppressive towards pathogenic microorganisms. Beneficial mycorrhizal fungi populations, which form an association with sugarcane roots, may also be affected.

Earthworms are commonly found in humid habitats and are probably one of the most important soil invertebrates (Toyota & Kimura, 2000). They are known to improve soil aeration and water infiltration by the tunnels they build in the soil (Ehlers, 1975). Earthworm casts increased growth and dry matter yield of maize and cowpea in Nigeria (Asawalam & Hauser, 2001) as they have high nutrient concentrations, more water-stable aggregates and higher organic carbon and nitrogen than soil without earthworm casts. In sugarcane fields on the north coast of KwaZulu-Natal, *Pontoscolex corethrurus* was prevalent in sugarcane rows than in interrows and were associated with sugarcane roots (Dlamini et al., 2001). Low values for organic carbon, microbial biomass carbon and earthworm numbers were recorded before pre-harvest burning and this reflect how soil organic matter loss occurs in sugarcane fields (Haynes & Hamilton, 1999). Higher earthworm numbers were recorded in green cane harvested fields with trash retention. Cadet et al.

(2002) found that sugarcane fields of KwaZulu-Natal, termites (*Macrotermes natalensis*) form mounds with typically high bacterial activities. The mounds are locally known as “isiduli”.

2.4 DISEASES AFFECTING SUGARCANE

Sugarcane rust is caused by two species of fungus, *Puccinia melanocephala* and *Puccinia kuenhnii* (Ryan & Egan, 1989). *Puccinia melanocephala* is found in almost all sugarcane producing areas and causes common rust in sugarcane that leads to extensive economic losses in many countries (Comstock et al., 1992, Raid & Comstock, 2000). Orange rust caused by *Puccinia kuenhnii* is limited to the Asia-Oceania region and causes serious losses in Hawaii, Fiji, Sumatra, Papua New Guinea and Australia (Magarey, 2000; Magarey et al., 2001). In Queensland, Australia, this disease caused a reduction in mean yield of sugarcane from 101.5 t/ha for the previous five years to 57.2 t/ha in 2000. In 2000, it was estimated that orange rust reduced production by 30 to 40 % in the Central, Burdekin, Herbert and Northern districts, which together make up 70 % of the Australian sugar industry. The loss in monetary terms was estimated at A\$100-140m to growers and A\$150-210m to the industry at large.

Ratoon stunting disease (RSD) caused by the bacterium *Clavibacter leifsonia* is the most damaging sugarcane disease in the world (Davis & Bailey, 2000; Hoy & Flynn, 2001). In South Africa, sugarcane losses over three crops were between 1 % and 41 % depending on the cultivar (Bailey & Bechet, 1997). In Louisiana, U.S.A., reductions in yield more than 50 % have been recorded in susceptible cultivars (Grisham, 1991). From 1997 to 2000, cultivars resistant to RSD (LCP85-384) have been grown in Louisiana and the number of infected stalks has decreased from 8 % to 0.4 % while the number of infected fields has decreased from 41 % to 6 % during the same period (Hoy & Flynn, 2001).

Yellow leaf syndrome (YLS) of sugarcane occurs in many countries including Australia, Hawaii, Brazil, Colombia, El Salvador, Guadeloupe, Guatemala, Malawi, Mauritius, Morocco, Reunion, South Africa, Swaziland, USA and Zimbabwe (Moutia & Saumtally, 1999; Grisham et al., 2001). This disease is caused by a luteovirus transmitted either by aphids and termed sugar cane yellow leaf virus (SCYLV) (Vega et al., 1997) or a phytoplasma transmitted by leafhoppers (Cronjé et al., 1998; Cronjé & Bailey, 1999; Rassaby, et al., 2000). The main symptoms are a yellowing of the abaxial part of the leaf midrib followed by yellowing of the leaf lamina (Comstock et al., 1999; Matsuoka & Meneghin, 1999; Lockhart & Cronjé, 2000; Moutia & Saumtally, 2001). In Louisiana, this disease was first discovered in 1996 and is spread by a sugarcane aphid, *Melanaphis sacchari* (Reagan et al., 2002). Results from two fields showed that yield and juice quality did not differ in SCYLV-infected and non-infected plant crops although yield from the first and second ratoons in SCYLV-infected crops was reduced by 11 and 14 % respectively. In Brazil, losses as high as 20-30 % in the susceptible variety SP71-6163 have been recorded (Comstock et al., 1994) while Vega et al. (1997) associated this disease with yield loss as high as 50 % in the SP73-6163 variety. Yellow

leaf syndrome first appeared in the South African sugar industry in 1994 and has now become common in all commercial varieties in most parts of the industry (Cronjé et al., 1998).

Sugarcane mosaic is caused by sugarcane mosaic virus (SCMV) and sorghum mosaic virus (SrMV) (Grisham, 2000). The symptoms of this disease are contrasting shades of green resulting from different chlorophyll concentration on the leaf blade. This virus has caused substantial losses to the South African sugar industry due to mosaic induced yield decline (Goodman et al., 1998). Under SCMV conditions, sucrose yield reduction has been reported to be as high as 42 % in a susceptible variety (Bailey & Fox, 1987). In Louisiana, this disease caused sugarcane yield losses ranging from 7 to 21 % over a three-year crop cycle.

Leaf scald, a disease caused by a bacterial pathogen, *Xanthomonas albilineans*, is another of the very important diseases of sugarcane (Saumtally et al., 1996) and has a worldwide distribution (Rott & Davis, 1996). It has recently appeared in epidemic proportions in Louisiana (Grisham & Legendre, 1993) and Mauritius (Autrey et al., 1995). This disease is transmitted mechanically by knives and harvesters and by planting infected setts (Saumtally et al., 1996). Leaf scald is a manageable disease and its impact can be minimised by using resistant sugarcane varieties (Mohamed et al., 1996, Rott & Davis, 1996).

In the tropics, yellow spot, a disease caused by a fungus *Mycovellosiella koepkei*, is an important leaf spot of sugarcane (Ricaud & Autrey, 1989; Autrey & Saumtally, 2000). This disease causes yellow spots on the surface of leaves, which may coalesce to cover large areas under humid conditions (Ramdoyal et al., 1996, 2001). In Thailand, this disease is widespread throughout the cane-growing areas with several sugarcane varieties such as F134, F137, Eros and Pindar showing susceptibility to it (Ouvanich & Srisink, 1996).

The most important fungal disease of sugarcane worldwide is smut caused by the fungus *Ustilago scitaminea* (McFarlane & McFarlane, 2002). Smut produces a whip-like sorus from lateral bud meristems of infected stalks (Comstock, 2000). The whips are made up of core of host tissue surrounded by a thin layer of black spores, which cause the disease. In Malawi, 85 % of sugarcane grown is variety N14 which has succumbed to smut (Isyagi & Whitbread, 2002) and in the Ord River Irrigation Area (ORIA) of Australia, it is regarded as a serious threat to the Australian sugar industry (Riley et al., 1999). Sugarcane varieties NCo310 and Q117 are most susceptible to smut and occupy about one third of the area planted.

In Queensland, *Pachymetra* root rot, which causes soft and flaccid rot of the primary shoot roots, is caused by *Pachymetra chaunorhiza* (Magarey & Croft, 1996). Pythium root rot caused by *Pythium arrhenomanes* is characterised by rotting of the root tips, reduction in root growth and root reddening with lesions (Rutherford et al., 2002). Some interactions between root pathogenic fungi and nematodes favour the presence of nematodes (Hasan, 1988). Infection of clover roots by fungal pathogen *Pythium* has been

shown to increase the densities of *Meloidogyne* spp. populations and severity of root-knot nematode symptoms (Zahid et al., 2002).

Papua New Guinea, as a centre of diversity of *Saccharum* spp., also has a diversity of pests and diseases associated with sugarcane (Magarey et al., 1996). Four new sugarcane diseases have been noted in Ramu, Papua New Guinea. Ramu stunt disease, a viral disease transmitted by planthoppers of the genus *Eumetopina* causes stunted sugarcane growth. Ramu Scorch disease causes severe browning and scorching of the shoots and leaves. Ramu orange leaf disease, a fungal disease caused by *Exobasidiales* spp., turns leaves from pale green to yellow and ultimately to orange and leads to shoot death. Ramu streak disease symptoms comprise thin yellow-green streaks on the leaf lamina.

2.5 NEMATODES AFFECTING SUGARCANE

Forty-eight genera and more than 275 nematode species have been recorded in and around sugarcane roots worldwide (Spaull & Cadet, 1990). At least five plant parasitic nematode species occur in any given sugarcane field (Stirling & Blair, 2000). Plant-parasitic nematodes can be grouped into three categories: ectoparasitic, endoparasitic and semi-endoparasitic nematodes (Ferraz & Brown, 2002). Ectoparasites are those forms whose bodies mostly remain on the soil while their long stylets pierce and feed on the root cells, e.g. *Xiphinema*, *Helicotylenchus*, *Paratrichodorus* and *Longidorus* spp. Endoparasites include root-knot nematodes (*Meloidogyne* spp.) and cyst nematodes (*Heterodera* and *Globodera* spp.). These two genera are sedentary endoparasites and induce feeding sites in the infected plant tissues. Semi-endoparasitic nematodes include *Pratylenchus* spp.

In Queensland, 35 species of plant-parasitic nematodes were associated with sugarcane, with *Pratylenchus zaei* being the most widespread (Stirling & Blair, 2001). The distribution of this species was not restricted by soil type as it was found as commonly in clay loam and heavy loam soils as in sands. Also found associated with sugarcane in Queensland were *Helicotylenchus dihystra*, *Xiphinema* spp., *Meloidogyne* spp., *Paratrichodorus minor*, *Tylenchorhynchus annulatus*, *Achlysiella williamsi*, *Rotylenchulus pavus* and *Criconemella* spp. In India sugarcane is associated with about 31 plant parasitic nematode genera with *Pratylenchus*, *Helicotylenchus*, *Tylenchorhynchus*, *Meloidogyne* and *Hoplolaimus* causing greatest damage to the crop (Mehta, 1992).

In South Africa, 90 species of 29 plant-parasitic nematode genera have been recorded (Spaull, pers. comm.) with *Pratylenchus*, *Helicotylenchus*, *Tylenchorhynchus*, *Meloidogyne*, *Xiphinema*, *Hoplolaimus* and *Paratrichodorus* being the most commonly associated with sugarcane. *Pratylenchus*, *Meloidogyne* and *Hoplolaimus* are endoparasitic nematodes found in the roots while *Helicotylenchus*, *Tylenchorhynchus* and *Xiphinema* are ectoparasites and are found in soil. *Meloidogyne* spp. occur more frequently in sandy soils

than in fine textured soils (Spaull, 1981a). This species causes galls on the tips of sett and young shoot roots and may also reduce the number of tillers. Twenty species of *Pratylenchus* have been recorded in sugarcane (Spaull & Cadet 1990). *Pratylenchus zeae* occurs in sugarcane worldwide and causes necrosis and lesions within the cortex of roots. It reduces shoot and root mass and stalk length as well as leaf yellowing. About 30 species of *Helicotylenchus* parasitise sugarcane, with *H. dihystra* being the most common. This genus feeds ectoparasitically or semi-endoparasitically in the root cortex and is a mild pathogen. Forty species of *Xiphinema* have been associated with sugarcane and feed on cells in the vascular tissues. Twenty-eight species of *Tylenchorhynchus* are associated with sugarcane and occur more frequently in sandy soils than in loam and clay soils. This genus feeds on epidermal cells and root hairs and causes necrosis and stunting of the lateral roots. Other nematode genera associated with sugarcane in South Africa are *Scutellonema*, *Criconemella*, *Hemicyclophora* and *Rotylenchulus*.

Although all plant-parasitic nematodes cause damage to plants, some can be categorised as severely pathogenic, e.g. *Meloidogyne*, *Pratylenchus*, *Paratrichodorus* and *Xiphinema* spp. and others as less pathogenic, e.g. *Helicotylenchus* and *Tylenchorhynchus* spp. (Stirling & Blair, 2001). In two studies conducted in West Africa and South Africa, *Meloidogyne* spp. and *Pratylenchus zeae* made up more than 75 % of endoparasites in the sett roots and they suppressed tiller development at both localities (Cadet & Spaull, 1985). In South Africa, *Xiphinema* and *Paratrichodorus* spp. were the dominant ectoparasites and caused shorter stalks and lower yield as a result of attacks on shoot roots that restricted water and nutrient uptake. In West Africa, *H. dihystra* was the dominant ectoparasite and it mitigated the pathogenicity of the other nematodes and hence yield was high. In KwaZulu-Natal, in the “isiduli” formed by termites, densities of *Helicotylenchus*, *Pratylenchus*, *Paratrichodorus* as well as free-living nematode populations were higher than in the surrounding areas (Cadet et al., 2002). *Meloidogyne* was absent while *Xiphinema* occurred in low numbers in the “isiduli”.

Plant-parasitic nematodes usually act as vectors of plant-pathogenic viruses or bacteria and occasionally pathogenic fungi as well (Riedel, 1988). The first evidence of this phenomenon was reported in 1958 when *Xiphinema index* efficiently transmitted grapevine fanleaf nepovirus to grapevines in California, USA (Ferraz & Brown, 2002). Species of *Xiphinema*, *Longidorus*, *Paralongidorus* are vectors of 12 nepoviruses whereas *Paratrichodorus* and *Trichodorus* spp. transmit three members of *Tobravirus*. The interaction between vector nematodes and their associated viruses is highly specific, with a certain nematode transmitting only a certain strain of virus and certain strain of virus being transmitted only by a particular nematode species.

2.6 IMPACT OF NEMATODES ON YIELD

Nematodes can suppress tillering within two to three months after planting (Cadet & Spaul, 1985). A study on two trials (one with *Meloidogyne* and another without) in KwaZulu-Natal showed that over a four-year period, *Meloidogyne* alone was responsible for 30 % of sugarcane loss, which is equivalent to 15 t/ha/annum (Cadet & Spaul, 2001). Revised estimates of crop loss from nematodes indicates a reduction in yield which has been estimated to be more than 1.6 million tons of cane per annum (Spaul & Cadet, 2003). In Bundaberg, Australia, nematodes reduce sugarcane yield by 30 –40 % in sandy soils (Magarey & Croft, 1996).

Many nematode species probably contribute to yield decline with the lesion nematode (*Pratylenchus* spp.) being the most important (Stirling & Blair, 2001). The destruction of the structural and fine roots, symptoms caused by *Pratylenchus* spp. are observed in all areas with yield decline syndrome. In Australia, yield decline costs the sugar industry between A\$200-300 million annually. In Zimbabwe, sucrose yields have declined from 1970 to 1996 despite access to chemical ripeners and irrigation (Donovan, 1999). In cases where a nematicide was not used, growing a tolerant variety led to increases in yield by between 25 to 124 % compared to the susceptible variety (Spaul & Cadet, 2003). In the study with termite mounds, *H. dihystra* occurred in high numbers within the mounds than elsewhere and those are places that had high sugarcane yields (Cadet et. al., 2002).

2.7 CONTROL MEASURES

As early as 1911, carbon bisulfide was used to kill soil nematodes (Ferraz & Brown, 2002). Nematicides are an effective means of controlling nematodes as they reduce the nematode population size (Spaul & Cadet, 1990) and ensure sustainable production (Cadet & Spaul, 1985, Spaul & Cadet, 2003). Application of nematicide at planting and to each of the following ratoons can increase sugarcane growth and improve yield (Cadet & Spaul, 2001). They are highly effective in sandy soils (Spaul, 1998) as was shown by the two studies in West Africa and South Africa where stalk population increased by 46 % in West Africa and by 21 % in South Africa while stalk length increased by 21 % in West Africa and by 35 % in South Africa (Cadet & Spaul, 1985). In Queensland, nematicide treatment reduced nematode population by 90 % and yield responses were greatest in sandy soils, which had high *Meloidogyne* spp. populations (Stirling & Blair, 2001). In KwaZulu-Natal, mean responses to high levels of aldicarb (11-15 kg/ha) were 11 % to 23 % in different seasons (Spaul, 1995). Aldicarb reduced numbers of *Paratrichodorus*, *Helicotylenchus* and *Pratylenchus* spp. (Spaul, 1997) and enabled plants to develop a sett root system similar to that of plants in fumigated soils (Pankhurst et al., 2001). Methyl bromide killed nematodes even when applied at lower concentrations (Ohr et al., 1996) and in Australia, sugarcane grown in fumigated soil had an early growth of primary and secondary shoots and large sett roots (Pankhurst et al.,

2001). Application of furfural at a concentration of 0.4 ml/L soil reduced *Helicotylenchus dihystra*, *Pratylenchus zeae*, *Xiphinema elongatum*, *X. mampara*, *Paratrichodorus* spp. and *Tylenchorhynchus* spp. numbers (Spaull, 1997). In greenhouse and microplot experiments, furfural suppressed *Meloidogyne* spp. (Rodriguez-Kabana et al., 1993). Other benefits of using nematicides are fast development of a full canopy that suppresses weeds and development of an extensive root system that leads to efficient nutrient and water uptake (Cadet & Spaull, 2003). On average, nematicides increased sustainable development by a factor of three in *Meloidogyne*-infested sites and by a factor of five in *Meloidogyne*-free sites.

Various microbes are antagonistic to plant parasitic nematodes and plant pathogenic fungi (Stirling, 1991). Microorganisms in the rhizosphere provide defence for roots against pathogen attack and are ideal as biocontrol agents (Weller, 1988). Some bacteria and fungi have been shown to be effective in controlling nematode pests (Kerry & Bourne, 1996). Bacteria destroy nematodes by their parasitic behaviour while non-parasite rhizobacteria reduce nematode populations by colonising the plant rhizosphere (Siddiqui & Mahmood, 1999). *Pasteuria penetrans* is an obligate parasite of nematodes with a wide range of hosts (Siddiqui & Mahmood, 1999). Application of this bacterium with nematode-opportunistic fungi was found to be beneficial in reducing nematode numbers. This bacterium not only prevents nematode reproduction by parasitising them but also reduces the infectivity of spore-encumbered juveniles. The rhizosphere bacterium, *Pseudomonas mendocina* inhibited reproduction of *M. incognita* (Duponnois, et al., 1999). According to these authors, the bacteria induced physiological modifications of the plant roots and that resulted in negative effect on the development of root-knot nematodes. Strains of bacteria, *Burkholderia cepacia* and *B. gladioli* showed antagonism towards soybean cyst and *Meloidogyne* spp. (Kloeper et al., 1992) while *Pseudomonas chlororaphis* was antagonistic to *Pratylenchus* spp. (Hackenberg et al., 2000). Vogel et al. (2002) showed that between 17 % and 29 % of *Burkholderia* spp. paralysed *Meloidogyne* juveniles.

Plant parasitic nematodes and vesicular-arbuscular mycorrhizae (VAM) occur together in the rhizosphere and therefore biologically interact with each other (Ingham, 1988). Interactions between nematodes and microorganisms may affect nematode population growth and survival. *Verticillium chlamydosporium* parasitised eggs of *Meloidogyne hapla* on lettuce (Viaene & Abawi, 2000), suppressed cereal cyst nematode populations (Kerry et al. 1982) and was also effective against *M. Hapla* and *M. incognita* on tomato (de Leij et al., 1993). These fungi limit plant parasitic nematode activity especially if the roots are colonised by the fungi before nematodes (Forge et al., 2001). Physiological changes brought about by VAM may make the root a poor food source for nematodes (Ingham, 1988). Mycorrhizae can prevent root disease caused by *Phytophthora* and *Pythium* species (Pozo et al., 2002). Also, they may create a physical barrier to pathogens, occupy niches and may produce protective antibiotics. In Kenya, the endophytic fungus (*Fusarium oxysporum*) reduced gall formation and *Meloidogyne incognita* populations in tomato plants (Hallman & Sikora, 1994).

Trichoderma harzianum is a soil saprophyte that can be used as biological control agents to protect plants from fungal pathogens (Knudsen et al., 1991, Harman, 2000, Rutherford et al., 2002). *Trichoderma* spp. showed an ability to colonise *Meloidogyne javanica* eggs and second-stage juveniles (Sharon et al. 2001). According to Mercer et al. (1992), *Trichoderma* spp. may degrade the walls of nematode eggs by producing combinations of chitinases and proteases. Stirling et al. (1998) found that *Arthrobotrys dactyloides* reduced galling caused by *M. javanica* on potted tomato plants by 57-98 %.

Entomopathogenic nematodes of the genera *Heterorhabditis* and *Steinernema* have a symbiotic relationship with bacteria of genera *Photorhabdus* and *Xenorhabdus* respectively (Han & Ehlers, 1999, Fallon et al., 2002). The symbiotic relationship between bacteria and entomopathogenic nematodes has been shown to be antagonistic to plant parasitic nematodes by Bird & Bird (1986). Symbiotic bacteria from entomopathogenic nematodes produce toxic metabolites (Chen et al., 1994) that are toxic to nematodes (Hu et al., 1999). According to Grewal et al. (1999), the interaction of *Steinernema* spp. with the allelochemical produced by *Xenorhabdus* spp. may be responsible for the antagonism towards *Meloidogyne* juveniles in tomato roots. *Photorhabdus luminescens*, a bacterial symbiont of *Heterorhabditis* spp., an entomopathogenic nematode (Jessen et al., 2000), produced indole, a natural product of plants, which showed toxicity to *M. incognita*, *Bursaphelenchus xylophilus* and some *Heterorhabditis* spp. (Hu et al., 1999). *Meloidogyne javanica* populations in tomatoes were suppressed by applications of entomopathogenic nematode *Steinernema glaseri* probably due to competition for space between the two nematode species since they all orientate to a carbon dioxide gradient along the roots (Bird & Bird, 1986). Ishibashi & Choi (1991) also demonstrated that numbers of *Meloidogyne incognita*-induced galls in tomato roots decreased with application of *Aphelenchus avenae* and *Steinernema carpocapsae*.

Plants live in close association with various microorganisms that can have detrimental effect on plant health by affecting nutrition and disease (Smith & Goodman, 1999). The plant genotype can affect microbial community populations and their composition. Some plants are resistant to plant parasitic nematodes (Cook & Evans, 1987). Certain combinations of microbes increase plant vigour or yield and reduce nematode populations or penetration on roots (De Leij et al, 1992, Duponnois et al., 1998). *Bacillus subtilis*, a bacterium and *Paecilomyces lilacinus*, a fungus, together increased tomato plant height and weight and suppressed numbers of root galls, eggs, juveniles and females (Gautam et al., 1995).

Some plants develop resistance or tolerance genes to counteract the attack and diversity of nematode species that can parasitise them (Page & Bridge, 1993). Nematode-resistant plants may resist during penetration, development or reproduction of the nematodes (Anwar & McKenry, 2000). Tolerance genes overcome infestation by pests by promoting the plant growth mechanisms while resistance genes inhibit the pests' reproduction. A study conducted on alfalfa and *Meloidogyne incognita* showed a resistance response of this plant to the nematode four days after root penetration (Potenza et al., 1996).

Several plants emit specific chemicals that make them unpalatable or repellent to herbivores and/or attract predators of the herbivores attacking the plants (Conlong & Kasl, 2001). Plants producing substances toxic to plant parasitic nematodes make these nematodes fail developing into adults and the nematodes may die prematurely before reproducing (Ferraz & Brown, 2002). Marigolds (*Tagetes* spp) grown in nematode-infested soil reduce nematode population levels and subsequently increase yield of the following crop (Siddiqui & Alam, 1988). African marigolds (*Tagetes erecta*) have also been shown to inhibit nematode reproduction (Ijani & Mmbanga, 1988, Kimpinski et al., 2000). A study conducted by Ploeg (2000) showed that *M. incognita* juvenile numbers were greatly reduced in tomato plants planted in soil previously planted with marigold. In Japan, marigold species are inter-cropped with watermelon and are antagonistic to *Pratylenchus penetrans* (Ferraz & Brown, 2002). Also, marigold managed to reduce *P. penetrans* numbers in tomato and potato treatments (Alexander & Waldenmaier, 2002). In Hawaii, three cover crops, marigold (*Tagetes patula*), yellow mustard (*Sinapis alba*) and Sunn hemp (*Crotalaria juncea*) showed that they were poor hosts of reniform nematode, *Rotylenchus reniformis* in pineapple fields (Wang & Sipes, 1997). Sunn hemp planted as a previous crop decreased the initial population of *P. zeae* (Mehta & Sundararaj, 1997). *Brassica* species are known to release toxic products and are used to suppress nematodes, weeds and soil-borne diseases (Mojtahedi et al., 1991). A glasshouse study showed that amending soil with broccoli reduced *M. incognita* and *M. javanica* numbers when temperature was between 30-35 °C for 10 days (Ploeg & Stapleton, 2001). Some sugarcane varieties are more tolerant to nematodes than others (Cadet & Spaull, 2003).

Another way of controlling plant parasitic nematodes is through fallowing, crop rotation, soil heating and use of organic amendments. As organic matter in soil increases, so does microorganism diversity and that may lead to microbial competition and antagonism against soil borne pathogens. Also, planting alternate crops increases soil suppressiveness. Success of biocontrol is greatly improved by the incorporation of certain types of organic matter that inhibit pathogen activity but support biocontrol agent activities (Hoitink & Boehm, 1999) e.g. use of filter cake at planting and green manures (Rutherford et al, 2002). Semi-tropical forage or medicinal legumes were evaluated as organic soil amendments (Walker et al., 1997). Dried tissues of *Desmodium*, *Leucaena*, *Senna* and *Sesbania* species significantly reduced number of galls on tomato plants by more than 50 %. Velvet bean (*Mucuna deeringiana*) is associated with antagonistic microflora in soil, leading to increase of *Bacillus* and *Arthrobacter* spp. and *Burkholderia cepacia* (Vargas-Ayala et al., 2000).

Organic amendments have been shown to be successful in nematode control (Mehta et al., 1994) especially those with high nitrogen content (Rodriguez-Kabana, 1986). Nematicidal activity of organic amendments in soil can be attributed to chemical mineralisation that leads to release of ammonia and toxic compounds from plant tissues, increase in nitrogen and carbon dioxide, and growth of fungi and bacteria antagonistic to nematodes (Morris & Walker, 2002). Muller & Gooch (1982) found that amending soil with oil cakes

brought down plant parasitic nematode populations. In a study conducted by Mehta & Sundararaj (1999), *Pratylenchus zae* populations in soil and roots were suppressed by application of oil cakes. The addition of organic amendments, botanical aromatics and rhizobacteria led to tomato plant-growth promotion and suppression of nematodes under greenhouse conditions (Martinez-Ochoa et al. 2001). Jaffee et al. (1993), found that application of organic amendments led to increases in numbers of bacteria, bacterivorous nematodes and nematode-trapping fungi.

Evidence shows that soil organic amendments can be used as cheap and easily available means of controlling nematode attacks on sugarcane roots and at the same time increase sugarcane yields as they are rich in nutrients.

CHAPTER 3

MATERIALS AND METHODS

3. 1 THE EFFECT OF TWO ECTOPARASITES, *Helicotylenchus dihystera* AND *Xiphinema elongatum* ON SUGARCANE GROWTH.

Ninety dm³ of soil were collected from La Mercy, a sugarcane farm on the North Coast of KwaZulu-Natal (29° 36' S -31° 6' E). The soil was collected along 200m rows from sampling points with high percentages of either *H. dihystera* or *X. elongatum*. Forty-five dm³ of soil were collected from sampling points with high *X. elongatum* densities and another 90 dm³ from sampling points with high *H. dihystera* densities. For high *X. elongatum* proportion, soil was collected from sampling points 11 and 16 in transect 1, 1 and 4 in transect 3 and sampling points 4 and 6 in transect 5 (Fig. 1). Soil with high *H. dihystera* percentages was collected from sampling points 37, 38 and 39 in transect 1, 38, 39 and 40 in transect 3 and sampling point 40 in transect 5.

The soil was put in 2.5 litre pots and subjected to six treatments with six replicates each. The treatments were:

- a) Soil with high *H. dihystera* proportions
- b) Soil with high *X. elongatum* proportions
- c) Mixed soil (a) and (b) - control
- d) Sterilised mixed soil + inoculated *H. dihystera*
- e) Sterilised mixed soil + inoculated *X. elongatum*
- f) Sterilised mixed soil – control

Equal amounts of 45dm³ soil with *H. dihystera* and *X. elongatum* were mixed together and sterilised. Six of the pots with sterilised soil were inoculated with *X. elongatum* (between 750 and 950 individuals per pot) and the other six pots were inoculated with *H. dihystera* (between 1300 and 1500 individuals per pot) two weeks after planting and again three months later (Fig. 1). The other six pots were not inoculated and acted as a control. The nematodes used for inoculation in this experiment had previously been inoculated in sterilised soil for four months. Three or four 8-10 cm deep holes were dug next to the roots and the water with nematodes introduced into these holes using a pipette. For the naturally-infested soil (non-sterilised soil), six pots were filled with soil with high proportions of *H. dihystera*, the other six with soil with high *X. elongatum* percentages while the other six were filled with mixed soil with high percentages of both species. A composite sample from the six pots per treatment was collected for nematode counting. Single-budded transplants of the N16 sugarcane variety were planted in each pot. The plants were watered

with 50 ml of water at 10h intervals by means of an automatic irrigation system. Shoot height measurements were taken monthly and the sugarcane growth rate was calculated per day.



Figure 1: Schematic diagram of the work programme from planting to harvesting.

Twelve months after planting, the sugarcane from six pots per treatment was harvested. Soil and root sub-samples from each of the pots were processed for nematode extraction (Seinhorst, 1962) and the genera enumerated under a microscope. The soil samples were left for two days in the trays with sieves covered with tissue paper acting as a filter while root samples were left in the mist-chamber for eight days to allow nematodes to wash out. The aerial and root biomass from each pot were measured after the leaves and roots had been dried in the oven for seven days. The data from the 36 pots was analysed by Analysis of Variance (ANOVA). The abundance of nematodes in sterilised soil treatments was calculated separately from the naturally infested soil. Only species that occurred in more than 10 % of the 36 pots were used in the analysis, viz. *P. zae*, *S. brachyurus*, *H. dihystra*, *Meloidogyne* spp., *Criconemella* spp., *X. elongatum*, *P. minor* and *Hemicycliophora* spp. The arcsin (square root of proportions) of the eight species was used to normalise the data.

The studies in sections 3.2 to 3.8 were conducted at a La Mercy sugarcane field on the north coast of KwaZulu-Natal. The rows of sugarcane that exhibited homogeneity in growth were chosen in order to eliminate the chances that the host plant may be responsible for differences in nematode communities. This is because the distribution of the roots of the host plant has an impact on the distribution of plant parasitic nematodes (Noe & Campbell, 1985). If the growth of individual sugarcane plants is not the same in a field, their root masses may differ and that may affect nematode numbers, depending on availability of space and food in the roots. In this study, the host plant had reproduced vegetatively, i.e., the genetic make-up was the same for the crop and therefore any change in nematode numbers would not have been attributable to genetic differences among the plants. If the plants were reproduced by fusion of two different gametes, the resistance of the plants to nematode attack would differ depending on whether the individual plants inherited weak or strong enough genes to withstand or counteract the attack.

3.2 NEMATODE DISTRIBUTION OVER THE CROP CYCLE (TIME).

A 200m row (transect) was chosen because the sugarcane growth along its length did not differ significantly, i.e., the individual plants were more-or-less of the same height. Forty sampling points, 5m apart, were marked along the transect where approximately 1000cm³ of soil and root samples were collected at a depth of 1-30cm with a spade and put in labelled plastic bags (Fig. 2). Sub-samples of 200cm³ of soil were processed according to the method of Seinhorst (1962) for ectoparasitic, endoparasitic and free-living nematode identification and counting. The samples were collected tri-monthly over a twelve-month period, i.e. there were four sampling dates: September 2000, November 2000, March 2001 and June 2001.

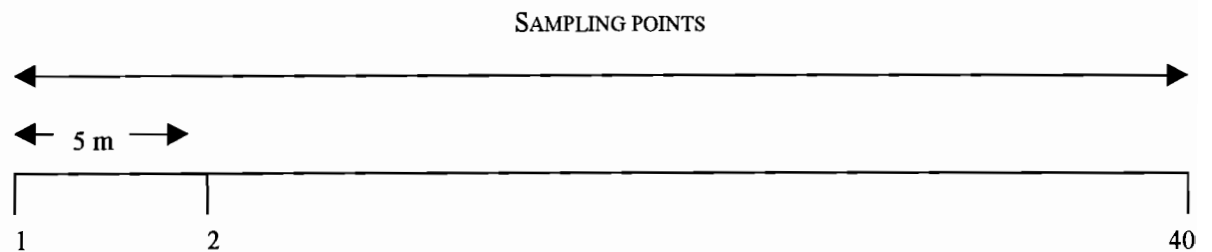


Figure 2: Diagrammatic representation of the 40 sampling points along the five transects.

The nematode data for the four sampling dates were analysed using ADE-4 software (Thioulouse et al., 1997) and Analysis of Variance. Free-living nematodes were not counted on the first samples collected in September 2000. Nematode abundance was studied to get their actual numbers in both soil and roots and to determine whether or not they were distributed evenly or in patches along the transect. The percentages were used to overcome the situation where large nematode numbers would display greater importance than low nematode numbers under the same conditions, whether in soil or roots.

The numbers of nematodes found per sampling point on the four sampling dates were used to determine if there was a change in nematode abundance along the transect. Species that occurred in more than 15% of the 40 sampling points per sampling date were used in the analysis. Seven species were found in more than six sampling points on each sampling date and were thus used in the analysis. Each data table had 40 rows (sampling points or individuals) and seven columns (nematode species or variables, viz. *Pratylenchus zeae*, *Scutellonema brachyurus*, *Helicotylenchus dihystra*, *Criconebella* spp., *Xiphinema elongatum*, *Paratrichodorus minor* and *Neodolichodorus brevistilus*) (Fig. 3).

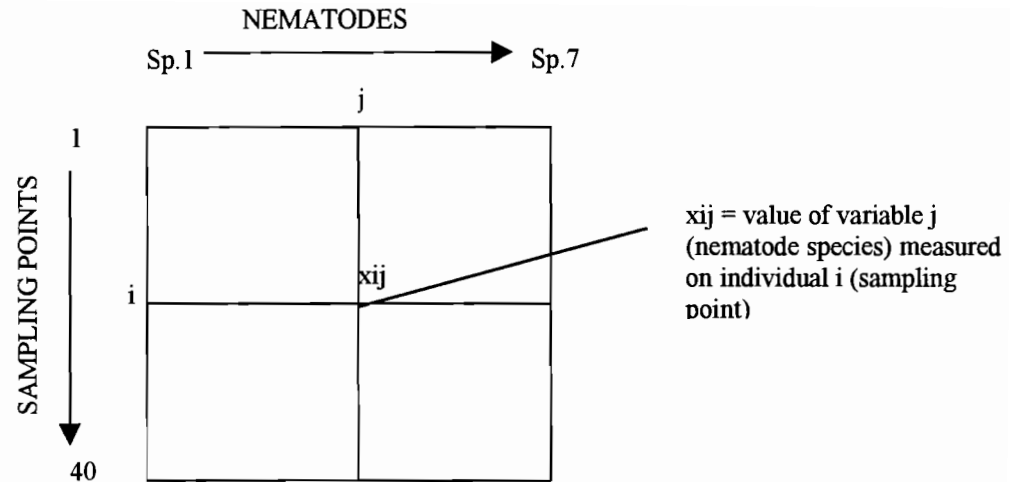


Figure 3: Table showing arrangement of rows (individuals) and columns (variables).

The nematode data for the four sampling dates were first analysed using the Three-Ways Table Analysis, a method that defines the common structure of several tables that share the same individuals and variables. The data from the four data tables were put one next to each other to form a single file with 40 rows and 28 columns (Fig. 4).

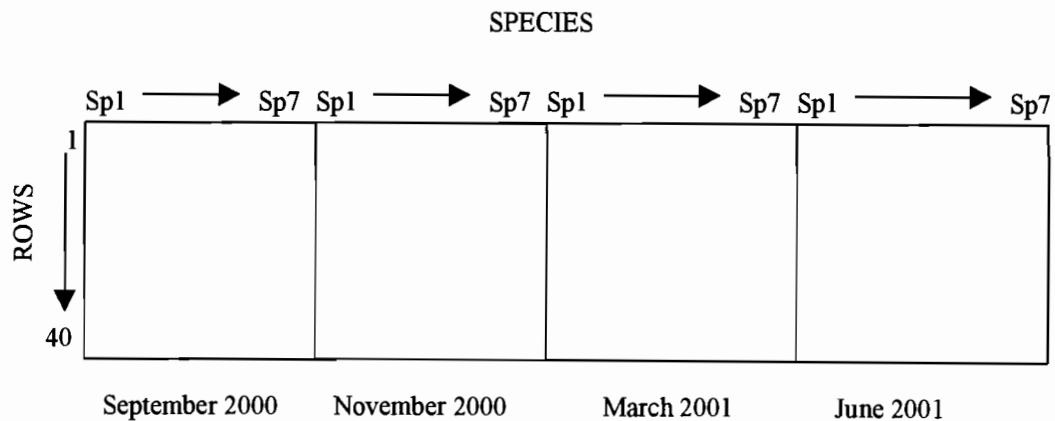


Figure 4: The four data tables arranged for Three-Way Table Analysis.

3.3 NEMATODE DISTRIBUTION ACCORDING TO SPACE.

Five adjacent 200m rows (transects) were chosen and the same procedure was followed as in 3.2 above for both soil and root processing as well as nematode data analysis. Instead of analysing data according to the four sampling dates, they were spatially analysed according to the five transects.

3.4 DISTRIBUTION OF ABIOTIC SOIL FACTORS ALONG THE FIVE TRANSECTS.

Soil samples were collected from the five transects as for nematode analysis in section 3.3. About 500 cm³ was analysed for physical and chemical properties: soil particle size, viz. silt, clay, fine sand, medium sand and coarse sand, pH, and levels (ppm) of soil elements, viz. P, K, S, Ca, Mg, Al, Na, Zn, Mn, and Fe (Barnard et al., 1990). The samples were collected once from each row, between July and August 2001.

The 11 soil element and five soil particle size data collected from the five transects were analysed using the Three-Ways Table Analysis, a method which defines the common structure of several tables that share the same individuals and variables. The soil element and particle data from the five transects were placed next to each other to form a single file with 40 rows and 80 columns. These 80 columns (5 X 16) for the soil elements and soil particles were compressed to 16 in the compromised table. This was to optimise the sum of the eigenvalues and show the importance of each element in relation to the first and second factors.

3.5 RELATIONSHIP BETWEEN NEMATODES AND ABIOTIC SOIL FACTORS.

The soil data file, initially had 200 rows from the five transects with 40 rows each. In this study, rows 26 and 27 were removed from each transect because they differed in their distribution from other rows when plotted on a compromised factorial map in the previous study because of their high soil element concentrations. The 11 soil elements and the five soil particle size datasets from the five transects were averaged per row to form one file with 38 rows and 16 columns. This was done to highlight those elements and particles that had significantly higher values than others.

Similarly, the nematode file was initially comprised of 200 rows as there were five data files (transects) with 40 rows each. Rows 26 and 27 were removed from each transect as was done for the soil data. In the five nematode files, only species that were present in more than 15% of the 38 sampling points per transect were used in the analysis. Seven species occurred in more than six sampling points in each transect, viz. *P. zaeae*, *Scutellonema brachyurus*, *H. dihystra*, *Criconemella* spp., *X. elongatum*, *P. minor* and *Neodolichodorus brevistilus*. The 190 rows of the five nematode files were averaged per species to make one file with 38 rows and seven columns. This was done to emphasize the importance of those species that occurred in either high or low proportions.

In order to determine whether there was a relationship between soil elements and nematode species along the five transects, coinertia analysis was run on the two datasets, i.e. the nematode and soil data. Coinertia explores the common structure of two tables that share the same statistical units. In this study, this will result in a factorial map of species linked to a factorial map of soil variables. The significance of cross matrix depends on the two separate analyses (PCA in this case). PCA and coinertia factor maps show which species and which variables are most interesting, i.e. those with different positions in the PCA factor map and in the coinertia analysis factor map (Cadet & Thioulouse, 1998). A permutation test, which shows whether a relationship between two tables is statistically significant or not, was computed. The rows of the two datasets are changed at the same time and after each permutation, the tables are centred and the matrix of the rows remains unchanged. This test shows that if one takes row X from Table 1, put it in front of row Y on Table 2 and run the test again, the relationship between variables in X and Y will remain as before the rows were interchanged. Scatter plots were then drawn to show whether there was a negative or positive relationship between certain nematode species and soil elements.

3.6 DISTRIBUTION OF LEAF ELEMENTS ALONG THE FIVE TRANSECTS.

Leaf samples were collected at 40 sampling points, 5m apart, from two 200m rows (transects 1 and 4). The third inner leaf blade was collected from each sampling point and analysed for elements (ppm), viz. N, P, K, S, Ca, Mg, Na, Zn, Mn, and Fe. The samples were collected once from each row, between July and August 2001. The leaf element data for the two transects were analysed using the ADE-4 software (Thioulouse et al., 1997). The 10 leaf element datasets collected from the two transects were analysed using the Three-Ways Table Analysis and PCA.

3.7 RELATIONSHIP BETWEEN NEMATODES AND LEAF ELEMENTS.

Nematode and leaf elements data from transects 1 and 4 were used in the analysis as leaves were collected from these two transects only. The leaf data file, initially, had 80 rows from the two transects with 40 rows each. Three rows were removed, rows 26 and 27 because they were removed from the nematode data, and row 1 because of high N values on leaf data in transect 4. A file with 74 rows and 10 columns was then created.

Initially, the nematode file was made up of 76 rows as there were two data files (transects) with 38 rows each since rows 26 and 27 were removed previously because of high soil element values. In this study, row 1 was also removed (as it was in the leaf dataset) because of high leaf element values. In the two nematode files, only species that were present at more than 15% of the 38 sampling points per transect were used in the analysis. The nematode file was then made up of 74 rows and seven columns (nematode species).

The nematode species and leaf elements were studied separately by Principal Component Analysis (PCA) on correlation matrix. In order to determine whether or not there was a relationship between leaf elements and nematode species along the five transects, coinertia analysis was run on the two datasets, i.e., the nematode and leaf data. A permutation test was also computed. Scatter plots were drawn to show whether there was a negative or positive relationship between certain nematode species and certain leaf elements.

3.8 RELATIONSHIP BETWEEN SOIL AND LEAF ELEMENTS.

In this section, only data from transects 1 and 4 were used as leaf data was collected from these transects only. Initially the soil data file had 190 rows from the five transects with 38 rows each, after rows 26 and 27 were removed because of the high element values in them. Here, the first row was also removed when coinertia analysis was performed between the soil and leaf element data. This was done after realising that the leaf element data in the first row had high element values, especially nitrogen. A soil data file with 16 columns (11 soil elements and five soil particles) and 74 rows from the two transects was then created. The leaf data file, had 74 rows from the two transects with 37 rows each after rows 1, 26 and 27 were removed, and 10 columns (leaf elements).

In order to determine whether or not there was a relationship between leaf and soil elements along the five transects, coinertia analysis was run on the two datasets, i.e. the soil and leaf data. A permutation test between the two datasets was also computed. Finally, scatter plots were drawn to show whether there was a relationship between soil and leaf elements.

3.9 MANIPULATION OF NEMATODE COMMUNITIES FOR LESS PATHOGENIC SPECIES THROUGH USE OF ORGANIC AMENDMENTS.

This study was conducted in a sugarcane field at Tinley Manor (31°12'E - 29° 30.25'S) on the North coast of KwaZulu-Natal. Seven treatments replicated seven times were randomly allocated along the field using Fisher blocks. The field was divided into seven blocks made up of seven plots each. Each plot was made up of five 10m long rows and the blocks were separated from each other by 2m breaks. Two hundred and forty-five rows were planted but only 147 rows (three middle rows in each plot) were harvested. Each plot was equal to 250 m² and about 90 m² ha of each plot were harvested. The seven treatments were:

- | | | |
|----------------------------|---|--------|
| i. Control | – | C |
| ii. Aldicarb | – | Ald |
| iii. Filter cake | – | F |
| iv. Filter cake + furfural | – | FF |
| v. Thume + filter cake | – | ThumeF |

- vi. Trash + filter cake – TrashF
- vii. Extra filter cake + furfural - BS

3.9.1 Planting

During planting, two whole sugarcane stalks were placed side by side in the furrow, top of one stalk to the base of the other stalk, then cut into setts with a cane knife and covered with soil. The method in which the eight treatments were applied was as follows:

3.9.1.1 Control – C

These plots were not treated with organic amendments. The planting method was as described in 6.2.1 above and only fertiliser was applied and the stalks were then covered with soil.

3.9.1.2 Aldicarb – Ald

Aldicarb-treated plots were used as reference treatments to measure the production potential. Planting procedure was the same as per above with 250g/ha of the nematicide used. Twenty-five grams/row of aldicarb were applied just before the setts were covered with soil. Aldicarb is highly toxic and evaporates easily and because of its toxicity and because the field had no covering vegetation, it was applied into the furrow where the nematodes live and where it would not be washed away by run-off after rain.

3.9.1.3 Filter cake – F

This is a by-product of sugarcane formed after sucrose has been extracted from the stalk and lime is added to the clarified juice, pressed and collected as solid cake (Dee et al., 2002). It is highly nutritious and promotes bacterial activity. It protects roots by releasing humic acid which is unappealing to nematodes. It does not have a structure thereby preventing nematodes from moving through the roots. Three hundred and thirty kg/ha of filter cake were used. Thirty-three kg of filter cake were applied in each 10m row, followed by placement of setts on it and then another layer applied on top of the setts. The setts were thus “sandwiched” between the filter cake layers so as to protect the roots from nematode attack. The calculations for amount of filter cake used in this study were:

49.6 kg of filter cake in a 75litre bin
 50l of filter cake applied per 10m row
 = 33 kg in 10m row
 1 hectare = 100m X 100m

$$\frac{100\text{m} \times 33 \text{ kg}}{10\text{m}} \\ 330 \text{ kg/ha}$$

3.9.1.4 Filter cake + furfural – FF

Filter cake and furfural were mixed together to determine if sugarcane yield would improve when organic matter was used in conjunction with a nematicide. Furfural has nematocidal properties and its active ingredient is 2-furfuraldehyde (Rodriguez-Kabana, 1993 et al.; Spaul, 1997). It is not only toxic to nematodes but also to sugarcane buds and therefore the filter cake was used to minimise its toxicity effect. Forty kg/ha of filter cake were mixed with 200 ml/ha of furfural (20ml furfural/1000 ml water dilution). Four kg of filter cake mixed with 20ml of furfural was applied to each 10m row, followed by the placement of the sett on top and then covered with soil.

3.9.1.5 Thume + filter cake – ThumeF

Thume is a by-product from paper-mills formed after the manufacture of paper and has no nutritional value. Four hundred and twenty-six kg/ha of thume and 66 kg/ha of filter cake were used. 42.6 kg/row of thume was applied followed by 6.6 kg/row of filter cake that acted as a bacterial starter and as a nutrient source, since thume is simply cellulose with no organic compounds in it. The setts were then placed on top, more thume applied over of setts to protect roots from nematode attack, then covered with soil. The calculations for thume and filter cake used were:

$$\begin{aligned} &42.6\text{kg of thume in a 75litre bin} \\ &42.6 \text{ kg of thume per 10m row} \\ &1 \text{ hectare} = 100\text{m} \times 100\text{m} \\ &= \frac{100\text{m} \times 42.6 \text{ kg}}{10\text{m}} \\ &= 426 \text{ kg} \end{aligned}$$

For filter cake:

$$\begin{aligned} &49.6 \text{ kg of filter cake in 75 litre bin} \\ &10 \text{ litre filter cake per 10 m row} \\ &= 6.6 \text{ kg filter cake in a 10 m row} \\ &\text{Therefore: } \frac{100\text{m} \times 6.6 \text{ kg}}{10\text{m}} \\ &= 66 \text{ kg/ha} \end{aligned}$$

3.9.1.6 Trash + filter cake – TrashF

Trash is the sugarcane leaves that are removed during harvesting. Filter cake was used to speed up the decomposition of trash and hence the release of nutrients. Thirty-six kg/ha of trash and 66 kg/ha of filter cake were used. 3.6 kg of trash per row was put on furrows and setts were placed on top. 6.6 kg of filter cake was then applied over the setts and then covered with soil. The calculations for the amount of trash used were:

$$\begin{aligned} & 3.6 \text{ kg of trash in 75 litre bin} \\ & 3.6 \text{ kg of trash in 10m row} \\ \text{Therefore } & \frac{100 \text{ m} \times 3.6 \text{ kg}}{10 \text{ m}^2} \\ & = 36 \text{ kg/ha} \end{aligned}$$

3.9.1.7 Extra filter cake + furfural – BS

A mixture of one litre of furfural mixed with 32 L/ha of water was used. Two hundred and fifty ml of furfural were mixed with eight litres of water and applied on two and a half rows using a watering can. Immediately after applying the mixture, 66 kg of filter cake were applied onto each row and then setts were placed on top. Calculations for the amount of furfural mixture and filter cake were:

$$\begin{aligned} & 250 \text{ ml of furfural in 25m} \\ \text{therefore } & \frac{100 \text{ m} \times 250 \text{ ml}}{25 \text{ m}} \\ & = 1000 \text{ ml} \end{aligned}$$

$$\begin{aligned} & 49.6 \text{ kg of filter cake in 75 litre bin} \\ & = 6.6 \text{ kg} \times 10 \\ & = 66 \text{ kg in 10m} \\ \text{therefore } & \frac{100 \text{ m} \times 66 \text{ kg}}{10 \text{ m}} \\ & = 660 \text{ kg/ha} \end{aligned}$$

3.9.2 Sampling

During the first three months after planting, soil samples (each 500 cm³) together with a sett with roots were collected at three, six, nine and 12 weeks from the guard rows (the rows on the outside on each plot) of each plot in alternation (Table 1). This was done to determine the effect of endoparasitic nematodes on sett roots during germination. Shoot roots were collected bi-monthly until harvesting and the effect of nematodes found in these roots on yield was determined.

DATE	TIME AFTER PLANTING	ACTIVITY
29 August 2000	0 weeks	PLANTING
18 September 2000	3 weeks	Soil and sett roots (samples pooled together)
09 October 2000	6 weeks	Soil, sett and shoot roots (samples pooled together)
30 October 2000	9 weeks	Soil, sett and shoot roots
21 November 2000	12 weeks	Soil, sett and shoot roots (samples pooled together)
05 January 2001	5 months	Soil and shoot roots
05 February 2001	6 months	Soil and shoot roots
02 April 2001	8 months	Soil and shoot roots (samples pooled together)
28 June 2001	11 months	Soil and shoot roots (samples pooled together)
20 September 2001	14 months	Soil and shoot roots (samples pooled together)
09 October 2001	14 months	HARVESTING

Table 1: Sampling timetable and activities for each sampling date. Samples pooled together according to treatments were not used in the analysis.

The soil and root samples collected in the first two and the fourth sampling dates were pooled together according to treatments while the samples for the third sampling date were processed according to plots. The non-pooled samples were used in the analysis. Both soil and root samples were processed for nematode extraction (Seinhorst, 1962) and the numbers in each genus counted under the microscope. The soil samples were left for 48 hours in trays over tissuepaper-covered sieves that acted as a filter while root samples were left in the mist chamber for eight days to allow nematodes to wash out of the roots. Infestation of shoot roots by endoparasites during the first three months after planting (germination period) and during the whole crop cycle until harvest were determined to check the effect nematodes on germination of shoots and their growth during the crop cycle. Correlations between the number of

endoparasites (average number found in sett root) and germination percentage of shoots, and between endoparasites and sett root weights was determined:

$$\text{Germination \%} = \frac{\text{Number of nodes with germinated shoots}}{\text{Total nodes in a sett}}$$

The yield data were analysed by Analysis of Variance (ANOVA). The nematode and yield data were analysed by Principal Component Analysis (PCA). Only species that occurred in more than 15% of the 56 rows were used in the analysis, viz. *Helicotylenchus dihystra*, *Scutellonema brachyurus*, *Xiphinema elongatum*, *Neodolichodorus brevistilus*, *Pratylenchus zae*, *Paratrichodorus minor*, *Tylenchorhynchus* spp., *Meloidogyne* spp., *Hoplolaimus* spp. and *Criconebella* spp. *Helicotylenchus dihystra* and *S. brachyurus* were counted together as one group as they have a close morphological resemblance even though *H. dihystra* occurred in higher proportions than *S. brachyurus*. As a result, they will be referred to as *H. dihystra* + *S. brachyurus* from now onwards. Effect of organic amendments on nematode community manipulation for the less pathogenic species was determined by projecting the 56 treatment plots on nematode rows. Yield data (tons/ha) from the three middle rows per plot were also projected on nematode rows to determine in which plots, dominated by which species, the yield was highest.

3.9.3 Harvesting

Twelve stalks from the three middle rows from all plots were taken for analysis of:

Fibre – part of cane not soluble in water, e.g. trash,

Brix – total of sucrose and water soluble impurities,

Purity – proportion of total soluble solids, i.e. sucrose,

Pol – same as sucrose,

Ash – non-combustible portion of dry matter and sand.

The weights of sugarcane from the three middle rows were added together per plot. Then, the weights from the seven replicates per treatment were added together and used to calculate the overall yield per treatment as follows:

$$\text{Yield} = \frac{Y \times \text{mass of cane}}{X \times 1000}$$

$$\text{Where } Y = \frac{10\,000}{\text{Row spacing (1m)}}$$

And X = number of rows x row length

$$= 21 \times 10$$

$$= 210$$

The weights from the guard rows were not used because roots from adjacent plots may have influenced the next rows and that may have had an influence on nematode abundance and sugarcane growth.

CHAPTER 4

THE EFFECT OF TWO ECTOPARASITES, *Helicotylenchus dihystera* AND *Xiphinema elongatum* ON SUGARCANE GROWTH

4.1 INTRODUCTION

Plant-parasitic nematodes are important agricultural pests (Cowgill et al., 2002) which depend on the host plant for survival (Page & Bridge, 1993). They are highly affected by the host plant (Yeates, 1999) as it determines the structure of the plant parasitic nematode community (Villenave & Cadet, 1999). They are closely linked to the growth of the host plant and are affected by any factor affecting the host plant. They also affect plant growth by interfering with the plants' symbiotic relationship with vesicular arbuscular mycorrhizae (Freckman & Caswell, 1985).

Plant-parasitic nematodes are important in soil ecological processes too and participate in many interactions affecting the crop plant and can directly or indirectly affect nutrient and water uptake in plants by their effect on root health (Stoffelen et al., 2000). They feed on both foliage and roots (Freckman & Caswell, 1985). Nematode abundance, together with the balance between species within the community, determines the extent of this damage (Villenave & Cadet, 1998). The way plants are parasitised may depend on the manner in which the nematode community is structured. Nematode communities dominated by *Tylenchorhynchus gladiolatus* and *Pratylenchus pseudopratensis* caused more damage to millet than communities dominated by *Helicotylenchus dihystera*, a weak pathogen (Villenave & Cadet, 1998). The same may have happened on sugarcane in West Africa (Cadet et al., 2001). In West Africa, the ectoparasitic community was dominated by *H. dihystera* while in South Africa the dominant ectoparasite was *X. elongatum* (Cadet & Spaull, 1985).

The study was conducted to examine the relative pathogenicity of communities dominated by either *H. dihystera* or *X. elongatum*. The hypothesis was that sugarcane with a nematode community dominated by *X. elongatum* shows poor growth while sugarcane with a nematode community dominated by *H. dihystera* grows faster and taller, resulting in good yield.

4.2 MATERIALS AND METHODS

See chapter 3, section 3.1.

4.3 RESULTS

Table 1 shows the initial nematode numbers, together with the percentages in which each species occurred in each treatment (put in brackets), from composite samples at the beginning of the experiment under the different treatments. Note that in the sterilised soil (control), a few *H. dihystra* individuals were found. In sterilised soil that was to be inoculated with *X. elongatum*, a few individuals of this species were found and in sterilised soil that was to be inoculated with *H. dihystra*, a few *H. dihystra* and *P. zae* individuals were found. These numbers were obtained from a 5ml sub-sample taken from a 40ml sample.

TREATMENTS	<i>P. zae</i>	<i>S. brachyurus</i>	<i>H. dihystra</i>	<i>Meloidogyne</i> spp.	<i>Criconemella</i> spp.	<i>X. elongatum</i>	<i>P. minor</i>	<i>Hemicycliophora</i> spp.
High <i>H. dihystra</i>	456 (32.8)	0 (0)	888 (63.8)	8 (1)	8 (1)	24 (1.72)	8 (1)	0 (0)
High <i>X. elongatum</i>	168 (45.7)	0 (0)	160 (43.5)	0 (0)	0 (0)	32 (8.7)	8 (2.2)	0 (0)
Sterilised – control	0 (0)	0 (0)	16 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Sterilised + <i>H. dihystra</i>	16 (66.7)	0 (0)	8 (33.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Sterilised + <i>X. elongatum</i>	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	8 (100)	0 (0)	0 (0)
Mixed - control	232 (36.7)	0 (0)	352 (55.7)	0 (0)	0 (0)	24 (3.8)	24 (3.8)	0 (0)

Table 1: Initial nematode species numbers and percentages calculated per row (in brackets) from composite samples (collected at the beginning of the experiment) of the different treatments.

The difference in the heights of sugarcane shoots in the different treatments was statistically significant, $p < 0.0001$. The shoots of sugarcane planted in sterilised soil inoculated with *H. dihystra* attained a greater height than sugarcane grown in sterilised soil inoculated with *X. elongatum*, sterilised soil with no nematodes inoculated (control) and soil with high *H. dihystra*, all of which showed no significant difference (Fig. 1). Sugarcane grown in pots with mixed soil (control) and that grown in soil with high *X. elongatum* percentages had a low growth rate and were not significantly different from each other.

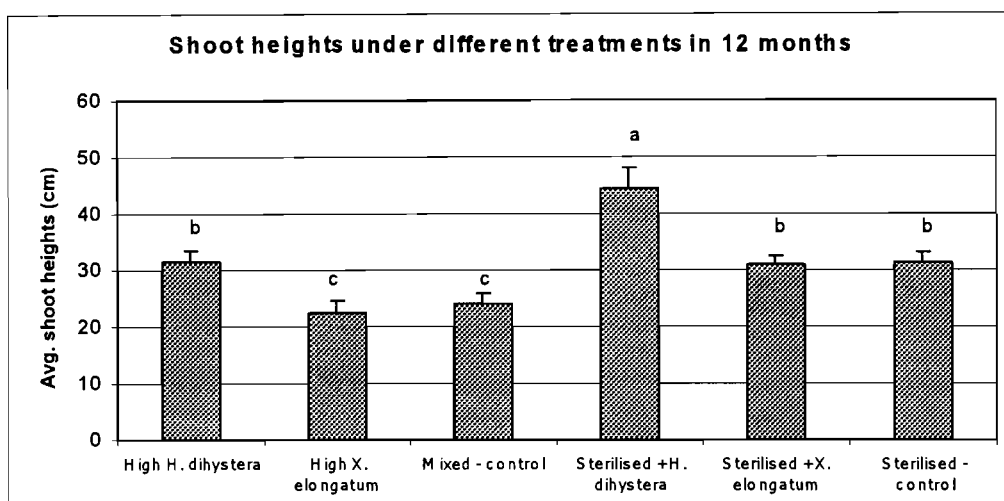


Figure 1: Differences in sugarcane shoot heights (cm) in soils with different proportions of *H. dihystra* and *X. elongatum* after 12 months. Bars with the same letter on top are not significantly different. Lines on top of bars represent standard error.

When the growth rate of shoot heights was determined (cm/day), the difference in shoot heights (cm) under the different treatments was found to be statistically significant, $p < 0.0001$. The means and standard error of the shoot growth rate are shown in Table 2. The growth rate of shoots per day was highest for sugarcane grown in sterilised soil inoculated with *H. dihystra*. The growth rates in sterilised soil inoculated with *X. elongatum*, sterilised soil (control) and naturally infested soil with high *H. dihystra* proportions did not differ much amongst themselves and were slower than that of sugarcane grown in sterilised soil inoculated with *H. dihystra*. Also, the growth rates of sugarcane grown in mixed soil (control) and in soil with high *X. elongatum* percentages showed no significant difference.

TREATMENT	Average growth rate (log) (cm/day)	Std. Error (log)
Sterilised + <i>H. dihystra</i>	0.111a	0.004
Sterilised + <i>X. elongatum</i>	0.094b	0.004
High <i>H. dihystra</i>	0.087b	0.006
Sterilised – control	0.084bd	0.004
High <i>X. elongatum</i>	0.072bc	0.006
Mixed - control	0.071cd	0.005

Table 2: Similarities and differences in mean sugarcane shoot growth rate (in logs) (cm/day) under six treatments. Means followed by the same letter are not significantly different ($p \leq 0.005$).

4.3.1. LEAF AND ROOT BIOMASS OF SUGARCANE GROWN UNDER DIFFERENT TREATMENTS

The difference in leaf biomass per treatment was statistically significant, $p \leq 0.0001$. For the sterilised soil, analysis of the leaf biomass data showed that sugarcane grown in sterilised soil inoculated with *H. dihystra* had more aerial biomass soil inoculated with *X. elongatum* and sugarcane grown in sterilised soil (control) which had less aerial biomass that were not significantly different (Table 3). For the naturally-infested soil, sugarcane grown in mixed soil (control) pots had more aerial biomass than sugarcane grown in soil with high *X. elongatum* percentages and soil with high *H. dihystra* percentages, which showed no significant difference between themselves.

The difference in root biomass for the six different treatments was statistically significant, $p \leq 0.0001$. Sugarcane grown in sterilised soil (control) and that grown in soil inoculated with *H. dihystra* had the highest root biomass while sugarcane grown in sterilised soil inoculated with *X. elongatum* had a low root biomass (Table 3). The root biomass of sugarcane grown in soil with originally high proportions of *H. dihystra*, high proportions of *X. elongatum* and mixed soil were not significantly different.

TREATMENT	Leaf biomass (g)	Std. error	Treatment	Root biomass (g)	Std. error
Sterilised + <i>H. dihystra</i>	42.15a	2.037	Sterilised + <i>H. dihystra</i>	6.84a	0.213
Sterilised + <i>X. elongatum</i>	25.75b	0.967	Sterilised – control	7.31a	0.215
Sterilised – control	23.62b	0.855	Sterilised + <i>X. elongatum</i>	5.36b	0.174
Mixed – control	17.8c	1.273	Mixed – control	4.43c	0.333
High <i>X. elongatum</i>	13.83d	1.198	High <i>H. dihystra</i>	3.82c	0.119
High <i>H. dihystra</i>	12.50d	0.809	High <i>X. elongatum</i>	3.78c	0.205

Table 3: Similarities and differences in mean leaf and root biomass of sugarcane grown under six different treatments with six replicates each. Means followed by the same letter are not significantly different ($p \leq 0.05$).

4.3.2 NEMATODE ABUNDANCE AND PROPORTIONS UNDER DIFFERENT TREATMENTS

The numbers of *H. dihystra* found in sterilised soil inoculated with this species were higher and significantly different from those found in sterilised soil inoculated with *X. elongatum* and the sterilised (control) soil, $p \leq 0.0001$ (Table 4). The *X. elongatum* numbers found in sterilised soil inoculated with this species were lower and significantly different from those recovered from the other two sterilised soil treatments, which showed no significant difference in numbers of this species recovered from them, $p \leq 0.019$. Numbers of *Hemicycliophora* spp. found in sterilised soil inoculated with *H. dihystra* were higher and significantly different from those found in the other two sterilised soil treatments, which had the same numbers of this species and thus a non-significant difference between them, $p \leq 0.0053$. For the other species (*P. zae*, *S. brachyurus*, *Meloidogyne* spp., *Criconebella* spp. and *P. minor*), the numbers recovered from both sterilised and naturally infested soil were not significantly different, $p \geq 0.05$.

TREATMENT	<i>H. dihystra</i>	<i>X. elongatum</i>	<i>Hemicycliophora</i> spp
Sterilised + <i>H. dihystra</i>	3.340a	0.159b	1.972a
Sterilised + <i>X. elongatum</i>	0.622b	1.138a	0.000b
Sterilised - control	0.000b	0.159b	0.698b

Table 4: Similarities and differences in nematode numbers (logs) recovered from sterilised soils inoculated with different species. Means followed by the same letter are not significantly different.

The numbers of *X. elongatum* recovered from the soil naturally infested with high numbers of this species were higher and statistically different from the other two treatments, $p \leq 0.014$. *Hemicycliophora* spp. numbers were higher in mixed soil (control) and significantly different from the other two treatments that were themselves not significantly different, $p \leq 0.0001$. *Scutellonema brachyurus* numbers found in naturally infested soil with high *X. elongatum* numbers were higher and significantly different from the numbers recovered from the other two treatments and which were not significantly different, $p \leq 0.006$.

TREATMENT	<i>X. elongatum</i>	<i>Hemicycliophora</i> spp	<i>S. brachyurus</i> spp.
High <i>H. dihystra</i>	1.611b	0.000b	1.181a
High <i>X. elongatum</i>	2.239a	0.159b	0.000b
Mixed - control	1.766b	2.246a	0.866a

Table 5: Similarities and differences in nematode numbers (logs) recovered from different naturally infested soils. Means followed by the same letter are not significantly different.

When the percentages of the individual nematode species found in the different treatments were analysed, some occurred in significantly different percentages in the different treatments while others did not show much difference in their percentages, e.g. *Meloidogyne* spp., *Criconebella* spp. and *P. minor* (Table 6). High percentages of *H. dihystra* were found in sterilised soil inoculated with *H. dihystra*. The percentages of *H. dihystra* found in soil with originally high *H. dihystra*, *X. elongatum* counts and mixed soil had no significant differences between them. Sterilised soil (control) and sterilised soil inoculated with *X. elongatum* had the lowest *H. dihystra* percentages. *Xiphinema elongatum* percentages were highest in sterilised soil inoculated with this species and in naturally *X. elongatum*- infested soil. The percentages of this species recovered from mixed soil and natural soil with high *H. dihystra* numbers did not differ much. Sterilised soil inoculated with *H. dihystra* and sterilised soil (control) had the lowest *X. elongatum* percentages. *Pratylenchus zeae* proportions were not significantly different in original soil with high *H. dihystra*, high *X. elongatum*, mixed soil and sterilised soil inoculated with *X. elongatum*. Also, sterilised soil (control) and sterilised soil inoculated with *H. dihystra* had more-or-less the same percentages of *P. zeae*. Numbers in brackets are the abundance of the nematode species under the different treatments.

Nematode species	H. dihystra	X. elongatum	P. zeae	S. brachyurus	Meloidogyne spp	Criconema spp.	P. minor	Hemicycliophora spp.
Treatments								
High H. dihystra	0.25b (7.68)	0.22ab (5.66)	0.51a (25.6)	0.15a (3.07)	0.03a (0.56)	0.04a (0.58)	0.83a (56.9)	0.00b (0.00)
Std. error	0.062	0.051	0.082	0.045	0.031	0.028	0.147	0.0
High X. elongatum	0.12b (1.78)	0.42a (18.24)	0.53a (27.9)	0.00bc (0.00)	0.00a (0.00)	0.01a (0.09)	0.80a (51.8)	0.01b (0.11)
Std. error	0.031	0.062	0.092	0.00	0.00	0.012	0.072	0.014
Mixed – control	0.24b (5.67)	0.26ab (7.18)	0.54a (26.9)	0.09b (1.21)	0.08a (1.25)	0.03a (0.30)	0.59a (31.8)	0.50a (25.5)
Std. error	0.021	0.04	0.061	0.029	0.036	0.021	0.073	0.101
Sterilised – control	0.00bc (0.00)	0.02b (0.34)	0.20a b (8.93)	0.00c (0.00)	0.00a (0.00)	0.00a (0.00)	0.51a (36.5)	0.32a (20.9)
Std. error	0.00	0.024	0.109	0.00	0.00	0.00	0.231	0.229
Sterilised + H. dihystra	1.03a (73.0)	0.01b (0.11)	0.09b (1.87)	0.00c (0.00)	0.02a (0.21)	0.16a (4.97)	0.31a (11.9 7)	0.25ab (7.86)
Std. error	0.046	0.013	0.045	0.00	0.019	0.73	0.078	0.065
Sterilised + X. elongatum	0.10bc (3.86)	0.56a (37.9)	0.45a (24.2)	0.03b (0.67)	0.03a (0.21)	0.09a (4.24)	0.49a (28.9)	0.00b (0.00)
Std. error	0.079	0.228	0.181	0.034	0.017	0.086	0.16	0.00

Table 6: Average proportions and abundance (in brackets) of nematode species in the six different treatments. Means followed by the same letter are not significantly different ($p \leq 0.05$).

4.4 DISCUSSION

Sugarcane grew better in sterilised soil than in naturally infected soil. Even amongst the sterilised treatments, soil inoculated with *H. dihystra* had better aerial and root growth than *X. elongatum*-inoculated soil (Table 7).

	TREATMENT	EXPECTED RESULTS	OBSERVED RESULTS
A	High <i>H. dihystra</i> proportions	Better growth with more root and leaf biomass.	Better growth than B, same leaf biomass as B, same root biomass as B & C
B	High <i>X. elongatum</i> proportions	Poor growth with less root and leaf biomass.	Poor growth, same leaf biomass as A, same root biomass as A & C
C	Mixed soil – control	Normal growth with less root damage due to <i>H. dihystra</i> mitigation.	Better growth than B, more leaf biomass than A and B, same root biomass as A & B
D	Sterilised soil - control	Best growth with more root and leaf biomass without nematodes to attack roots.	Better growth, less leaf biomass than E but same root biomass
E	Sterilised soil + <i>H. dihystra</i>	Better growth, more root and leaf biomass than in soil inoculated with <i>X. elongatum</i> .	Best growth, more leaf and root biomass than F but same as D
F	Sterilised soil + <i>X. elongatum</i>	Less growth, less root and leaf biomass than in soil inoculated with <i>H. dihystra</i> .	Less growth, less root and leaf biomass than in D and E

TABLE 7: Expected and observed results of the growth of sugarcane on the different soil treatments.

The better growth of shoots and the higher growth rate of sugarcane grown in sterilised soil inoculated with *H. dihystra* than of sugarcane grown in sterilised soil inoculated with *X. elongatum* and sterilised soil (control) shows that *H. dihystra* is a less pathogenic ectoparasite, causing less damage to the shoot roots than *X. elongatum* does. Naturally infested soil with high percentages of *H. dihystra* produced better growth than soil with high *X. elongatum* percentages and mixed soil (control), showing that *H. dihystra* is a weak pathogen. Damage caused to roots by *X. elongatum* may lead to inefficiency in water and nutrient uptake which in return interferes with plant growth (Spaull & Cadet, 1990). The low growth rate of sugarcane in soil with *X. elongatum* as the dominant ectoparasite supports the hypothesis that sugarcane

grown where there are high proportions of *X. elongatum* exhibits poorer growth than in soil where *H. dihystra* is the dominant species.

The greater aerial biomass of sugarcane grown in sterilised soil inoculated with *H. dihystra* than in sterilised soil inoculated with *X. elongatum* and the non-inoculated soil also proves that the plant stalks and leaves received adequate water and nutrients from the roots. The greater aerial biomass of sugarcane planted in mixed soil than that of sugarcane in soil with high *X. elongatum* and *H. dihystra* percentages proves that *H. dihystra*, when found co-occurring with other species in a community, has a mitigating effect on the other species. Roots form the least known part of the soil-plant-atmosphere continuum and yet they are important in supplying the plant with water and nutrients to ensure a successful crop (van Antwerpen, 1999). The non-significant differences in root biomass of sugarcane planted in non-inoculated sterilised soil and in sterilised soils inoculated with *H. dihystra* shows that even though *H. dihystra* is an ectoparasite, its effect on roots is not as severe as in those attacked by *X. elongatum*. The low root biomass in sterilised soil inoculated with *X. elongatum* shows how pathogenic this species is in relation to *H. dihystra*. In high *H. dihystra*, high *X. elongatum* and mixed soils, the non-significant difference in root biomass agrees with the hypothesis that the presence of *H. dihystra* in a nematode community neutralises the pathogenicity of the other plant parasitic species.

The occurrence of species like *Criconebella*, *P. zae* and *P. minor* in high proportions in sterilised soil inoculated with *H. dihystra* and *X. elongatum* respectively may be due to inadequate sterilisation of the soil. The non-significant difference of *H. dihystra* proportions in all treatments except in sterilised soil inoculated with this species may be due to interspecific interactions in the naturally infested soil as species reproduce. Nematode species may interact directly, due to competition for food and space within a root or when one species influences the suitability of the environment for colonisation by other species (Freckman & Caswell, 1985). That may lead to an increase or a decrease of one or some species within the community. This applies to *X. elongatum*, where proportions of this species showed no significant difference in all treatments except in sterilised soil inoculated with the species and the naturally infested soil that had high proportions of the species.

CHAPTER 5

WITHIN-FIELD VARIATION OF NEMATODE SPECIES ACCORDING TO TIME AND SPACE IN KWAZULU-NATAL

5.1 INTRODUCTION

The abundance of nematodes depends on the presence of a host plant (Cadet & Thioulouse, 1998; Page & Bridge, 1993) and is important in determining the structure of the plant parasitic nematode community (Villenave & Cadet, 1999). In sugarcane fields, nematode communities are generally made up of large numbers of endoparasitic and ectoparasitic species (Spaull, 1981), as well as free-living nematodes. Endoparasitic nematodes, e.g. species of *Pratylenchus* and *Meloidogyne* feed on the vascular tissues of the cortical parenchyma while ectoparasitic nematodes, e.g. *Helicotylenchus dihystra* feed on the epidermis of the roots (Bernard, 1992).

A nematode species may occur in different proportions in different environments and at different times (Ricklefs, 1987). This was in fact observed in sugarcane fields in West and South Africa where nematode communities were dominated by *H. dihystra* in W. Africa while in S. Africa they were dominated by *X. elongatum* and *Paratrichodorus minor* (Spaull & Cadet, 1990). In Burkina Faso, endoparasite numbers were high in sett roots during the plant crop but lower in stool (a stump or rootstock that produces shoots) and shoot roots during the same period (Cadet & Spaull, 1985). Nematodes, like all animals, reproduce and multiply over time, leading to variation in abundance within the communities. As a result, the proportions of the different nematode species in a community are expected to change from time to time.

Nematodes show patchiness at scales of 1 to 100m (Thornton & Matlack, 2002) and their distribution is usually clustered around a food source (Bernard, 1992). Competition amongst the different species for food and space may lead to an increase or a decrease of some species, causing the nematode numbers to change within the community. In a sugarcane field, sugarcane plants are exactly the same, as they develop vegetatively from setts and therefore the nematode numbers are not expected to change as the food source is the same, unless if the volume of the food source differs, i.e. the sugarcane growth is different.

Crops planted in a field have a common feature of uneven growth, with poor growth areas next to good growth areas (Cadet et al., 2001). Sugarcane is propagated vegetatively and therefore its genetic make-up is the same and its growth should also be the same. Its fields usually exhibit patchy growth however, which can be attributed to nematode parasitism, interaction with other soil organisms and soil heterogeneity. A study conducted by Noling & Ferris (1985) on alfalfa plants showed that micro-plots infested with *Meloidogyne hapla* caused continuous plant death than those without, creating patches which led to

reduction in yields. *Pratylenchus scribneri* and *Hoplolaimus galeatus*, the soybean nematode pests, are found in patches in soybean fields (Alby et al., 1983).

The interaction between nematodes and other soil organisms can lead to patchiness as was demonstrated by the interaction between entomopathogenic nematodes, *Steinernema* and *Heterorhabditis* spp. and their symbiotic bacteria *Xenorhabdus* and *Photorhabdus* in controlling the vine weevil, *Otiiorhynchus sulcatus* (Long et al., 2000; Samaliev et al., 2000). The bacterial symbionts carried by the entomopathogenic bacteria kill the insect host whose attacks on the vine plants would lead to patches along the field due to poor growth. In a study conducted in California, *Pratylenchus neglectus* was found to suppress yields in sandy soils but not in clay soils (Umesh & Ferris, 1994).

A. DO NEMATODE COMMUNITIES IN A SUGARCANE FIELD CHANGE DURING THE CROP CYCLE?

If, as in this study, the host plant has the same genetic make-up throughout the field, can nematode abundance and proportion change, even slightly, over time as the plants grow?

5.2 MATERIALS AND METHODS

The whole sampling procedure, materials used and the way data was analysed are described in Chapter 3, section 3.2.

5.3 RESULTS

5.3.1 QUANTITATIVE CHANGES

5.3.1.1 NEMATODE ABUNDANCE ALONG THE TRANSECT PER SAMPLING POINT

When data for ectoparasitic nematodes, viz, *Helicotylenchus dihystrera*, *Xiphinema elongatum*, *Neodolichodorus brevistilus*, *Paratrichodorus minor* and *Criconebella* spp. was analysed using Analysis of Variance, the difference in the distribution of the ectoparasitic nematodes along the transect was found to be statistically significant, $p \leq 0.03$. The change in abundance of ectoparasitic nematodes per sampling point along the transect for the four sampling dates showed that the nematode numbers were lower on the first half of the transect and higher over the second half of the transect (Fig. 1a). When data for free-living nematodes was analysed, there was no significant difference in the distribution of these nematodes along the transect, $p \geq 0.54$ (Fig. 1b).

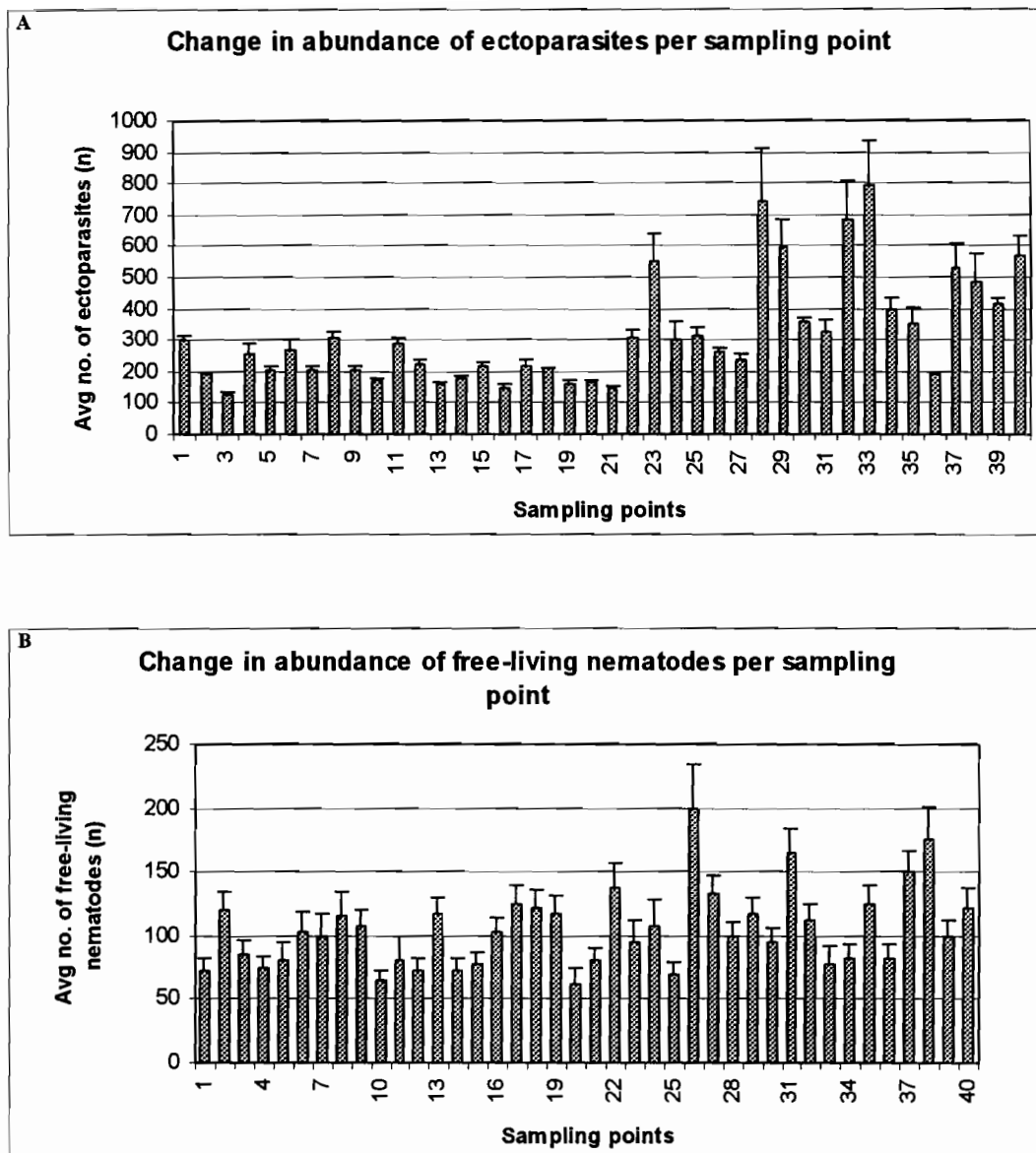


Figure 1: Change in nematode abundance per sampling point for the four sampling dates, (A) ectoparasites in soil, (B) free-living nematodes in soil.

When data of endoparasite found in the roots was analysed, the difference in endoparasite numbers found in each sampling point for the four dates was not statistically significant ($p \geq 0.93$) (Fig. 2).

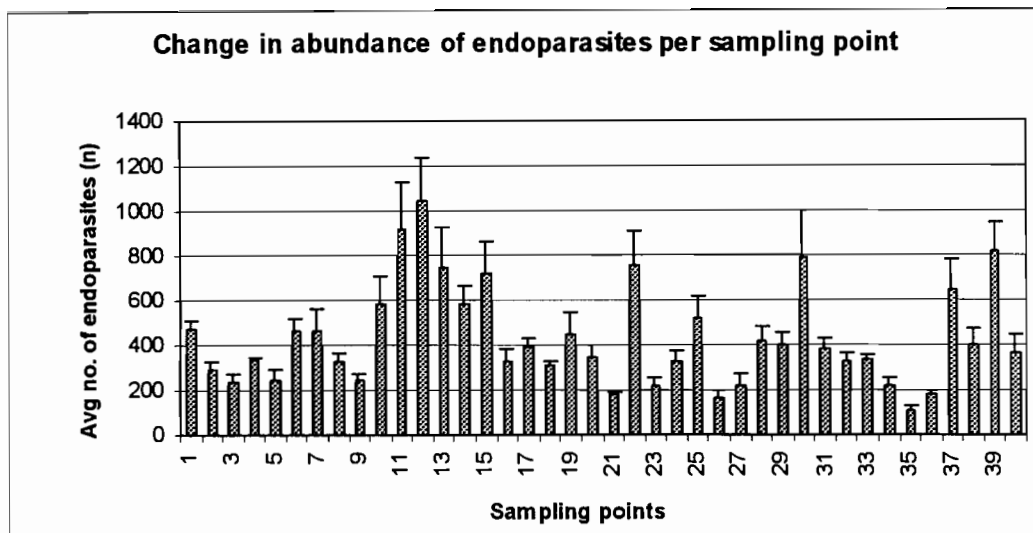


Figure 2: Change in endoparasite abundance in roots per sampling point for the four dates.

5.3.1.2 NEMATODE ABUNDANCE ALONG THE TRANSECT PER SAMPLING DATE

The change in ectoparasite abundance between some of the sampling dates was statistically significant, $p \leq 0.0001$ (Table 1). High numbers of ectoparasites were found on the 40 soil samples collected on the second sampling date and were significantly different from the percentages of ectoparasites collected on the other three sampling dates. The first, third and fourth sampling dates showed no significant difference in numbers of ectoparasites found in their soil samples. The log of the average number and standard error of ectoparasites found on each sampling date are also shown.

	Average number (log) of ectoparasites/200 cm ³ of soil	Standard error (log)
September 2000	2.259b	0.034
November 2000	2.595a	0.063
March 01	2.323b	0.025
June 2001	2.367b	0.032

Table 1: Similarities and differences of ectoparasite abundance (in logs) in 40 soil samples on each of the four sampling dates. Means followed by the same letter are not significantly different.

The average numbers of free-living nematodes found in 200 cm³ of soil per sampling date are shown in Table 2. Only data from three sampling dates were analysed as free-living nematodes were not counted on

the first sampling date. The change in free-living nematode numbers amongst the three sampling dates was statistically significant, $p \leq 0.0001$.

	Average number (log) of free-living nematodes/200 cm ³ of soil	Standard error (log)
November 2000	2.249a	0.029
March 2001	1.939b	0.042
June 2001	2.049c	0.038

Table 2: Similarities in numbers (in logs) of free-living nematodes found in 40 soil samples on each of the three sampling dates. Means followed by the same letter are not significantly different.

The average numbers of endoparasites found in roots during each sampling date are shown in Table 3. The change in endoparasite numbers between some sampling dates was statistically significant, $p \leq 0.0001$. Endoparasite percentages found in root samples collected on the second sampling date were significantly higher than of those collected on the third sampling date. The first and fourth sampling dates had low percentages of endoparasites in their roots and showed no significant difference between them.

	Average number (log) of endoparasites/ g of roots	Standard error (log)
September 2000	2.028c	0.055
November 2000	2.916a	0.052
March 2001	2.469b	0.044
June 2001	2.111c	0.058

Table 3: Differences and similarities in endoparasite abundance (in logs) in 40 root samples on each of the four sampling dates. Means followed by the same letter are not significantly different.

5.3.2 QUALITATIVE CHANGES

Relative percentages were used to check if there was a change in the composition of nematode communities per sampling point and their distribution along the transect during the four sampling dates. Percentages minimise the differences between nematode counts, and because it is not the actual number of nematodes that counts but the proportions in which they occur in a community, percentages were used.

Three-ways Table Analysis was performed on the nematode variables. The twenty-eight columns (4 X 7) for the nematode species were compressed to seven in the compromised table so as to optimise the sum of the eigenvalues and show the importance of each species in relation to the factors. The compromised factorial map of the nematode variables described 38.4% and 20% of the variability for the F1 and F2 axes respectively (Fig. 3). The variables corresponding to *H. dihystra* and *X. elongatum* had high positive and negative F1 factorial values respectively. Along the F2 axis, the variables corresponding to *P. zae* showed a strong correlation with the positive values of this factor and opposed to *X. elongatum*, *P. minor*, and *Cricnemella* spp. *Neodolichodorus brevistilus* and *S. brachyurus* occurred almost at the centre of the factorial map, with very low factorial values.

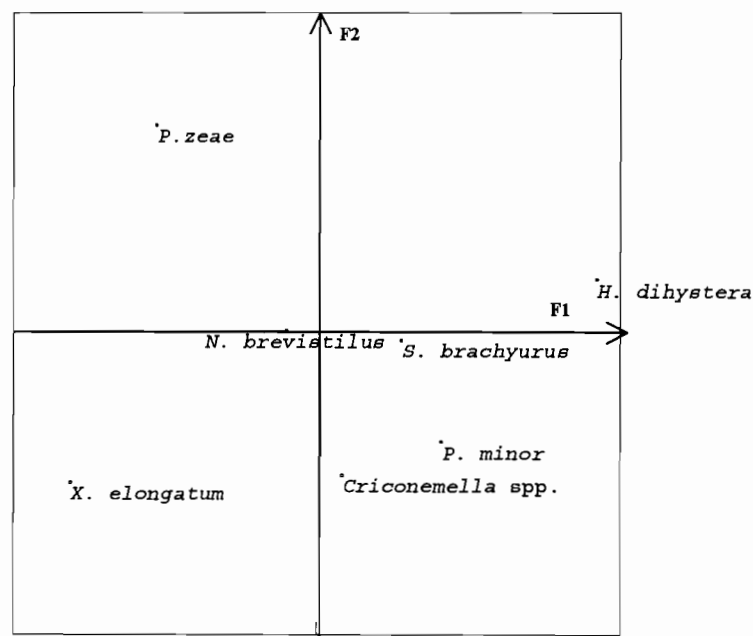


Figure 3: Compromised factorial map (F1 X F2) of the Three-ways Table Analysis on nematode variables for the four sampling dates. F1 = first factor, F2 = second factor.

The distribution of individual nematode species shown in the compromised factorial map in Fig. 3 is also demonstrated in Fig. 4 but according to all four sampling dates. For the four sampling dates, all the variables corresponding to *P. zae* and *X. elongatum* had negative factorial values as indicated by their occurrence on the negative half of the F1 axis. All the variables corresponding to *H. dihystra* had positive factorial values as shown by their occurrence on the positive half of the F1 axis. The aggregation of the points corresponding to the four sampling dates for each species means that the nematode communities did not change much over the sampling period.

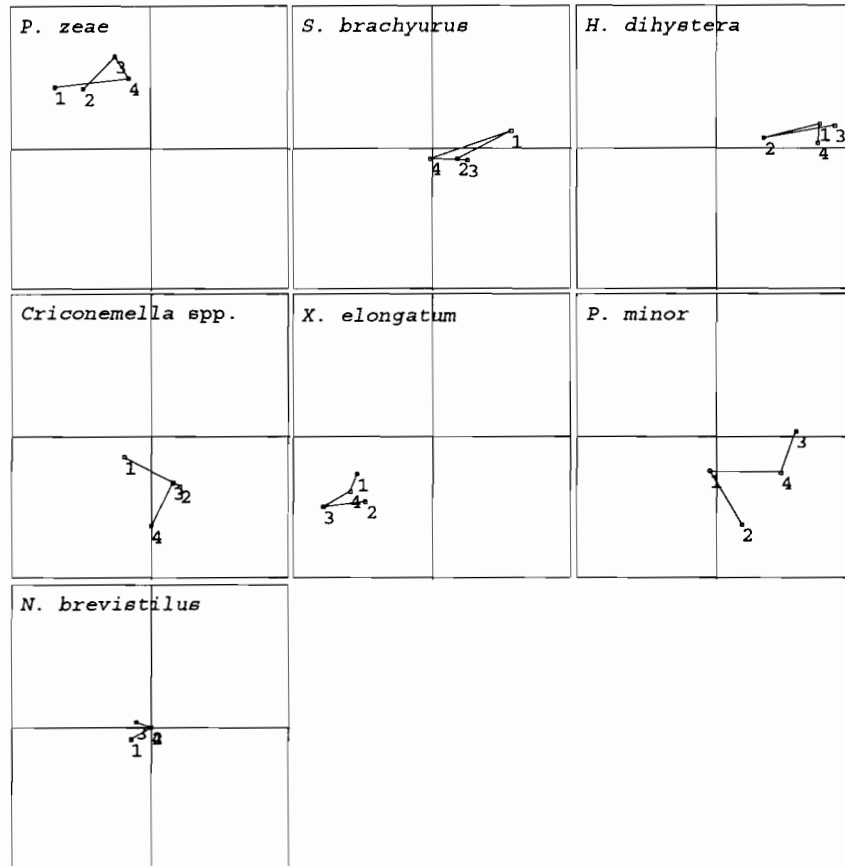


Figure 4: Projection of the four sampling dates for each nematode species on the factorial plan.
(1= 1st date, 2= 2nd date, 3= 3rd date, 4 = 4th date).

The factorial values of the 40 points corresponding to the samples collected along the transect were projected on the map of the transect instead of on the F1 X F2 factorial plan (Fig. 5). Two homogenous zones were clearly demarcated at the extremes of the F1 factor map where the first half was dominated by squares and the second half dominated by circles. This demarcation showed similarity of values for the nematode species per sampling point in the two zones. The circles, corresponding to the positive factorial values, represented a nematode community dominated by *H. dihystra* (Fig. 5). The squares, corresponding to negative factorial values, represented a nematode community dominated by *X. elongatum*. For F2, there was an alternation of areas with circles and squares although this pattern was not strong. To check if there was a difference in nematode distribution along the transect, the percentages of the three important nematode species were plotted per sampling point for the four sampling dates (Fig. 6).

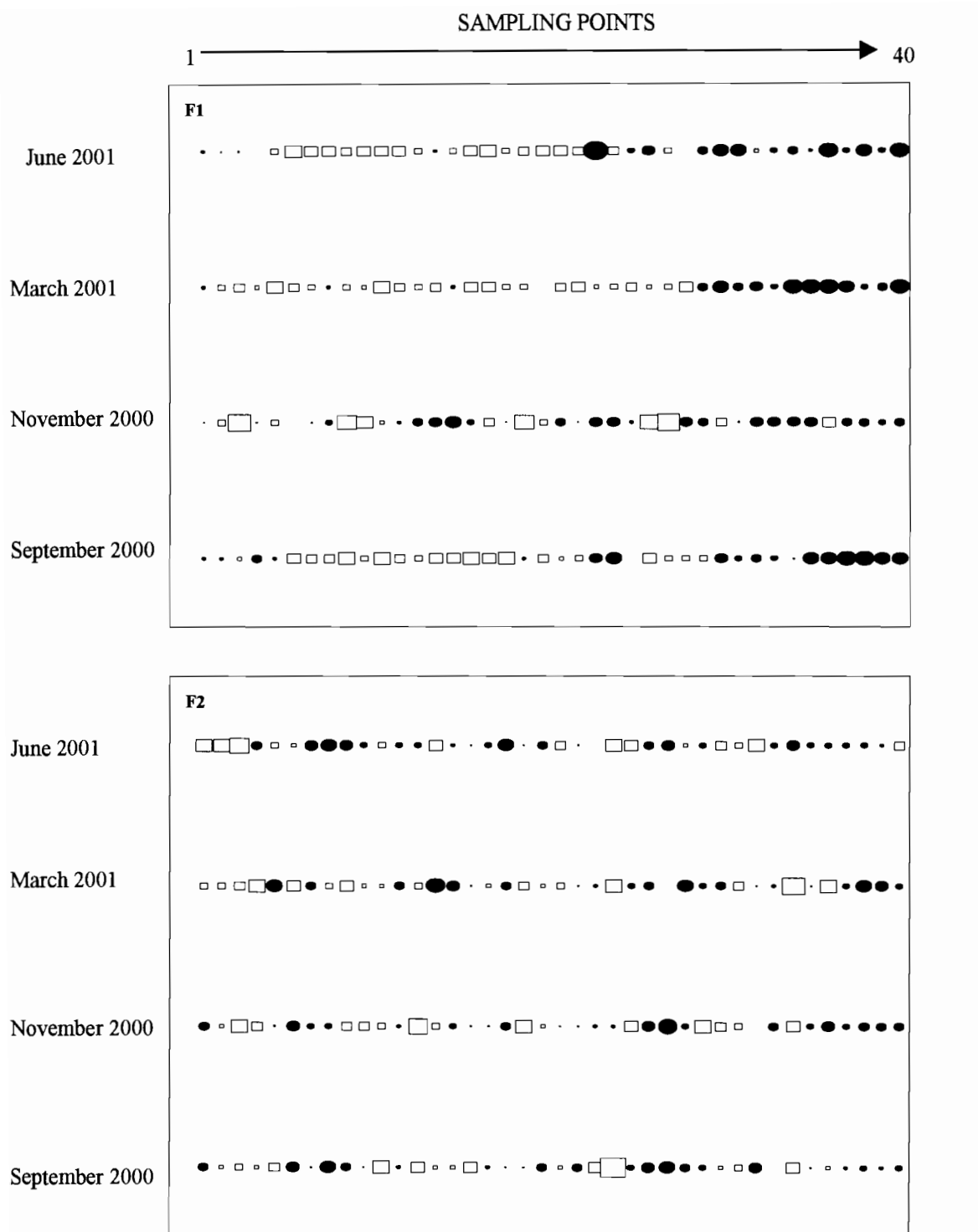


Figure 5: First and second factorial values of the Principal Component Analysis on the relative percentages of the nematode species projected on the trial map. Circles correspond to positive factorial values and squares to negative values. Size of the symbol is proportional to the absolute value.

When the percentages of *X. elongatum*, *H. dihystra* and *P. zae*, were plotted along the transect, a difference in the distribution of the percentages of the first two species along the transect was observed. High percentages of *X. elongatum* occurred in the first half of the transect while high percentages of *H. dihystra* occurred in the second half of the transect (Fig. 6). *Pratylenchus zae* did not show any consistent pattern from one sampling point to another or between dates.

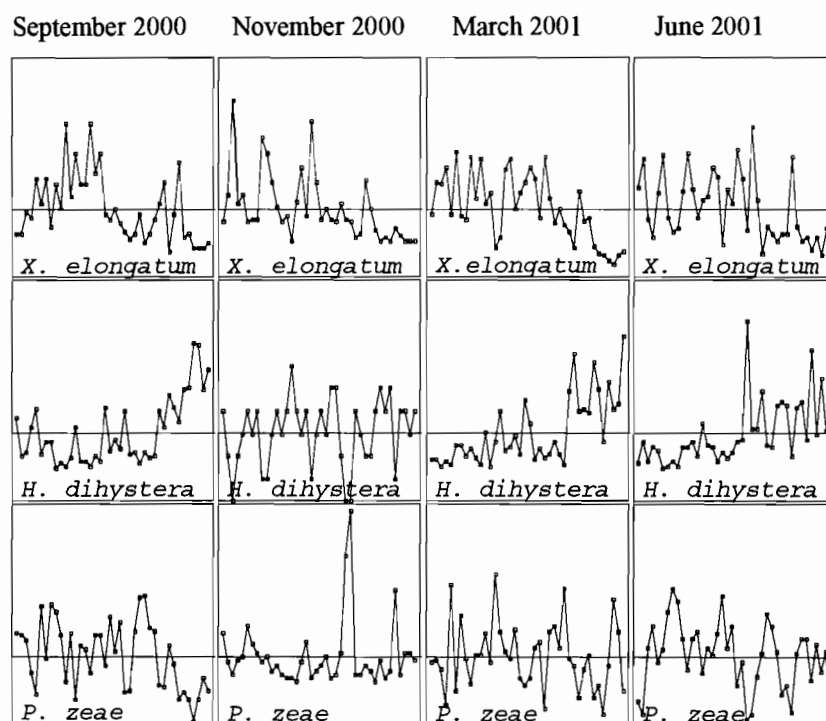


Figure 6: Change of relative percentages of the three important nematode species (*X. elongatum*, *H. dihystra* and *P. zae*) along the transect on the four sampling dates. The middle line represents the average in each case.

When percentages of *H. dihystra* were plotted against percentages of *X. elongatum* for all the sampling dates combined, the correlation coefficient issued had a negative value (Fig. 7). The regression line cut the y-axis at 35.6 % and the calculated x-value was 42.4 %. The correlation coefficient, $r = 0.72$, showed a strong correlation between *X. elongatum* and *H. dihystra*. The regression coefficient, slope = 0.84 was negative, meaning that as *H. dihystra* percentage increased, *X. elongatum* percentage decreased.

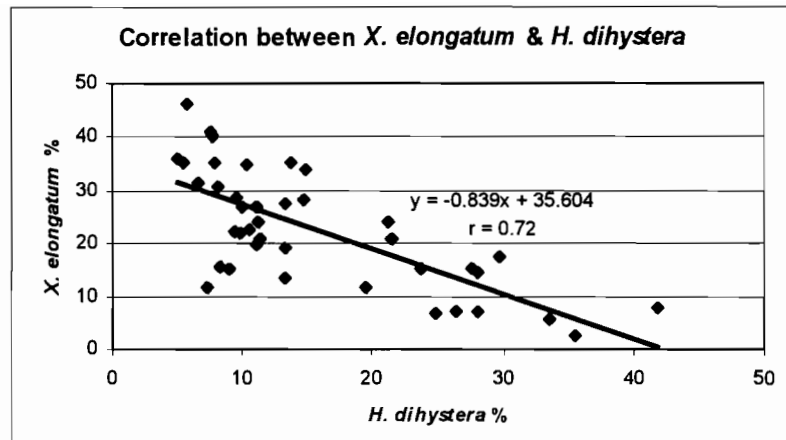
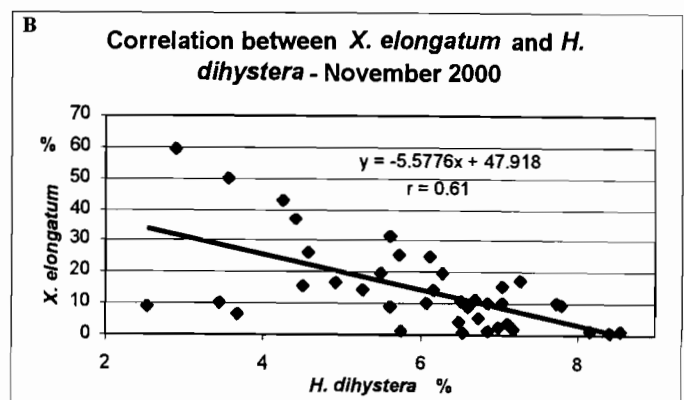
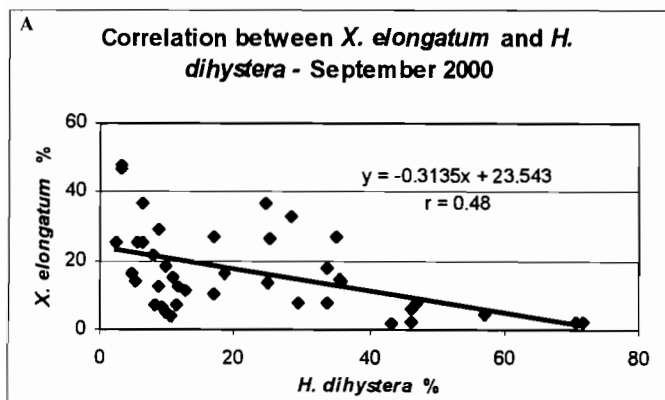


Figure 7: Significant negative regression between *X. elongatum* and *H. dihystra* for the four sampling dates.

When the analysis of the proportions of *H. dihystra* were plotted against those of *X. elongatum* per sampling date, the correlation coefficients were negative (Figs. 8 A-D). For all four sampling dates, the regression coefficient was negative implying that *H. dihystra* percentages increased as *X. elongatum* percentages decreased. For the first sampling date, the regression line cut the y-axis at 23.54 % while the calculated x-intercept was 75.1 % (Fig. 8a). The correlation coefficient, $r = 0.48$, showed that there was a strong correlation between *X. elongatum* and *H. dihystra*. For the second sampling date, the y-intercept was at 47.92 % and the x-intercept was 8.59 % (Fig. 8b). The correlation coefficient, $r = 0.61$, showed that the correlation between the two nematode species was strong. For the third sampling date, the regression line cut the y-axis at 44.5 % while the x-intercept was equal to 64.6 %. The correlation coefficient was equal to 0.59, showing a strong correlation between the two species. For the fourth sampling date, the y-intercept was at 36 % and the x-intercept at 65 %. The correlation coefficient, $r = 0.44$, showed a strong correlation between the two nematode species.



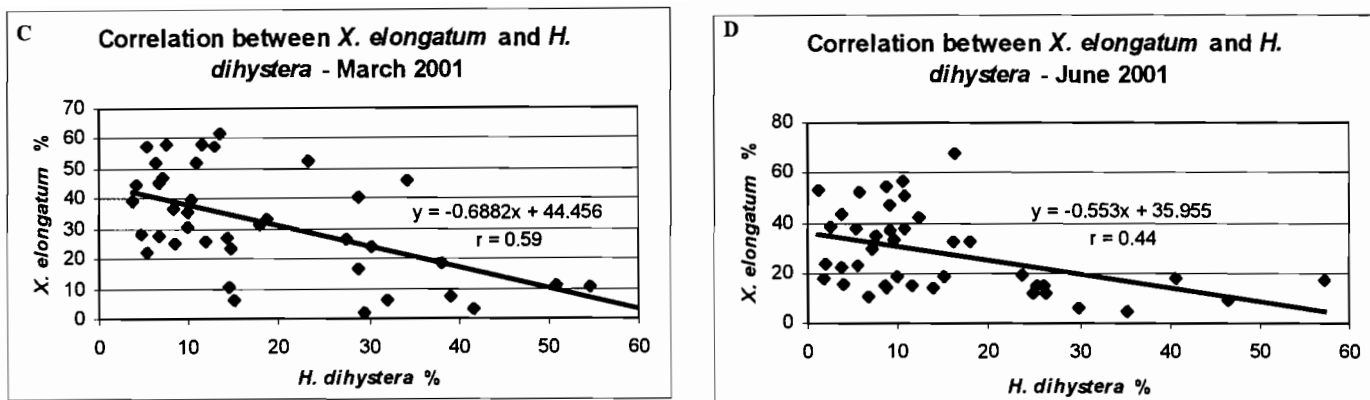


Figure 8: Significant negative regressions between *X. elongatum* and *H. dihystra* for the four sampling dates.

B. DOES SPATIAL DISTRIBUTION OF NEMATODE COMMUNITIES CHANGE ALONG A SUGARCANE FIELD?

In section A of this study, the nematode communities remained stable over the crop cycle although they differed in their distribution along the transect, with high numbers of *X. elongatum* found on the first part of the row while high numbers of *H. dihystra* occurred on the second half of the row. The nematode distribution was therefore explored further by determining whether it was a chance occurrence or whether it occurred in other field rows located in the immediate vicinity as well?

5.4 MATERIALS AND METHODS

The sampling procedure and data analysis are described in Chapter 3, section 3.3.

5.5 RESULTS

5.5.1 QUANTITATIVE CHANGES

5.5.1.1 NEMATODE ABUNDANCE ALONG THE FIVE TRANSECTS PER SAMPLING POINT

The numbers of ectoparasites found in the different sampling points along the five transects did not show much variation in their distribution (Fig 9a). Analysis of variance showed that there was no significant difference in the distribution of nematodes per sampling point along the five transects ($p \geq 0.28$). The numbers of free-living nematodes found in the different samples also showed a uniform distribution along the five transects ($p \geq 0.1$), showing a non-significant difference in numbers amongst the five transects (Fig. 9b).

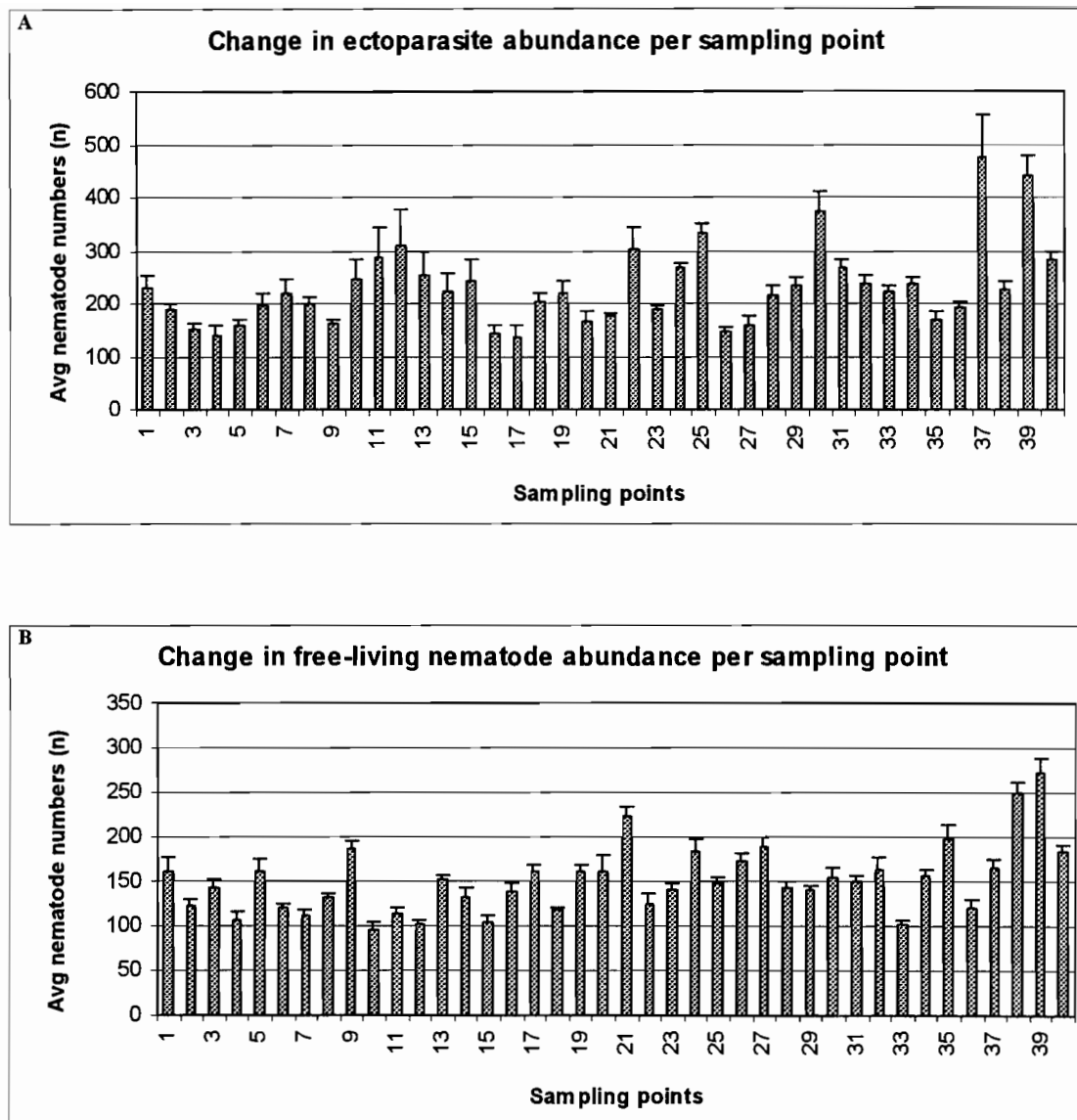


Figure 9: Change in nematode distribution at the 40 sampling points along the five transects, (A) ectoparasites in soil, (B) free-living nematodes in soil. Bars represent standard error.

So too, the numbers of endoparasites found in the different samples along the transects did not show much difference in their distribution ($p \geq 0.78$) (Fig. 10). Even though the endoparasite numbers differed from one sampling point to the next, they were not significantly different.

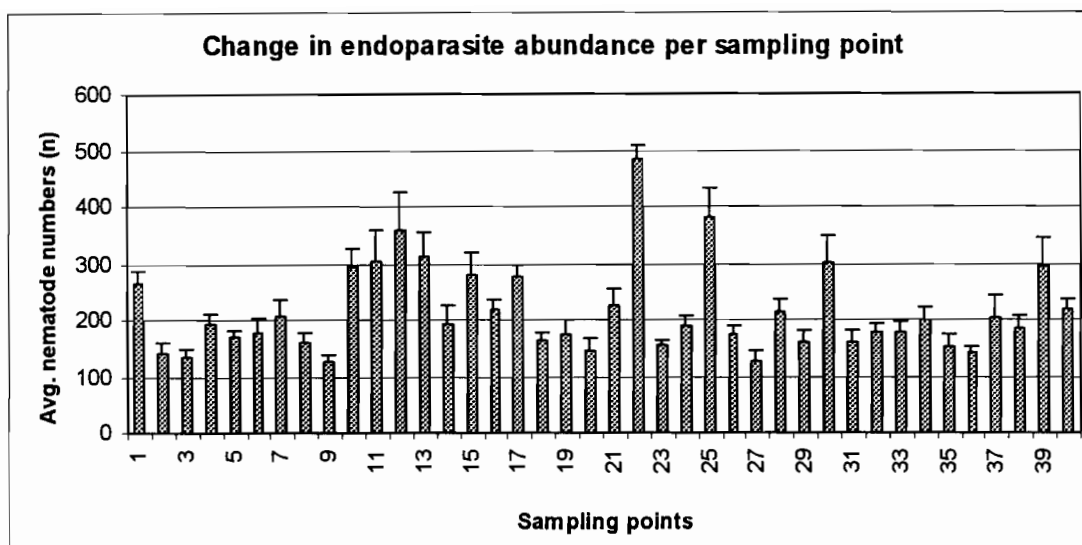


Figure 10: Change in endoparasite abundance at the 40 sampling points along the five transects. Bars represent standard error.

5.5.1.2. NEMATODE ABUNDANCE ALONG THE FIVE TRANSECTS PER SAMPLING DATE

Differences in the abundance of ectoparasitic nematodes between transect 1 and the other transects were statistically significant, $p \leq 0.0001$. The ectoparasite numbers found in transect 1 were higher and significantly different from ectoparasite numbers found in the other four transects, which showed no significant difference amongst themselves. The average numbers, standard deviations and standard errors of ectoparasites found in each transect are shown in Table 4.

	AVERAGE NUMBER (LOG) OF ECTOPARASITES/200 CM ³ OF SOIL	STANDARD ERROR (LOG)
Transect 1	2.581a	0.035
Transect 2	2.144b	0.036
Transect 3	2.180b	0.029
Transect 4	2.230b	0.036
Transect 5	2.233b	0.039

Table 4: Similarities and differences in numbers (in logs) of ectoparasites found in 40 soil samples along each of the five transects. Means followed by the same letter are not significantly different.

The average numbers, standard deviations and standard errors of free-living nematodes found along each transect was determined and are shown in Table 5. The differences in free-living nematode numbers between some transects were statistically significant, $p \leq 0.0001$. Transect 4 had the highest free-living nematode numbers and were significantly different from those in transect 2, which had a non-significant difference between itself and numbers in transects 3 and 5. Transect 1 had the lowest numbers of free-living nematodes and was significantly different from the other four transects.

	AVERAGE NUMBER (LOG) OF FREE-LIVING NEMATODES/200 CM ³ OF SOIL	STANDARD ERROR (LOG)
Transect 1	2.003d	0.019
Transect 2	2.127bc	0.037
Transect 3	2.167ac	0.027
Transect 4	2.229a	0.035
Transect 5	2.124c	0.040

Table 5: Similarities and differences in numbers (in logs) of free-living nematodes found in 40 soil samples along each of the five transects. Means followed by the same letter are not significantly different.

The average numbers, standard deviation and standard error of endoparasites found per transect were determined and are shown in Table 6. The change in endoparasite numbers between some of the transects was statistically significant, $p \leq 0.0001$. Average number of endoparasites found in roots collected from the 40 sampling points in transect 1 was significantly higher than of endoparasites in transect 5. Average endoparasite numbers in transects 2 and 4 were lower than those in the above-mentioned transects but showed no significant difference between themselves. Transect 3 had the lowest average endoparasite numbers in its root samples.

	AVERAGE NUMBER (LOG) OF ENDOPARASITES/GRAM OF ROOTS	STANDARD ERROR (LOG)
Transect 1	2.581a	0.035
Transect 2	2.054c	0.050
Transect 3	1.851d	0.054
Transect 4	2.112c	0.047
Transect 5	2.345b	0.035

Table 6: Similarities and differences in numbers (in logs) of endoparasites found in 40 root samples along each of the five transects. Means followed by the same letter are not significantly different.

5.5.2. QUALITATIVE CHANGES

Three-Ways Table Analysis was performed on the nematode variables. The 35 columns (5 X 7) for the nematode species were compressed to seven in the compromised table so as to optimise the sum of the eigenvalues and show the importance of each species in relation to the first and second factors. The compromised factor map of the nematode variables described 53.4 % variability of the F1 axis and 16 % variability for F2 axis. The variables corresponding to *X. elongatum* and *P. zae* had high negative F1 factorial values while variables corresponding to *H. dihystra* and *P. minor* had high positive F1 factorial values (Fig. 11). The variables corresponding to *S. brachyurus* had high positive factorial values along the F2 axis. *Neodolichodorus brevistilus* and *Criconebella* spp. occurred almost at the centre of the factorial map and had low factorial values.

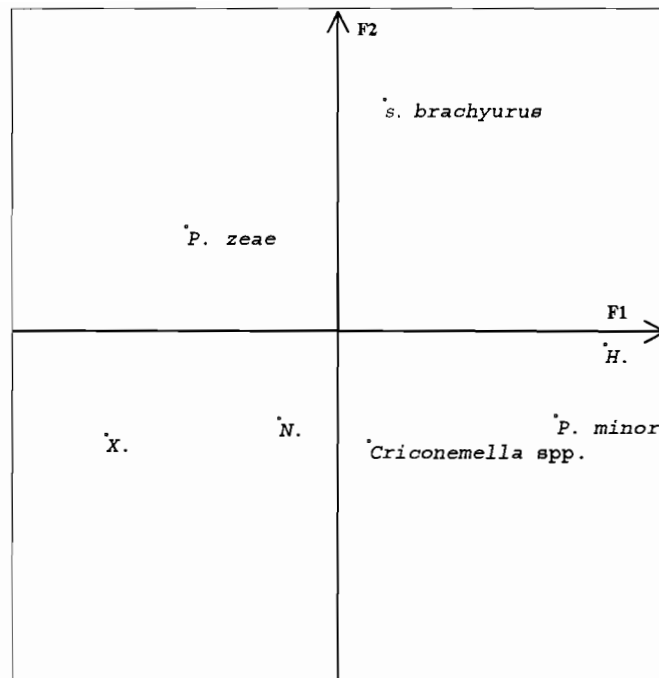


Figure 11: Compromised factorial map (F1 X F2) of the Three-ways Table Analysis on nematode variables for the five transects.

The distribution of nematode species shown in Fig. 11 was also demonstrated in Fig. 4 according to the five transects. For the five transects, all variables corresponding to *P. zae* and *X. elongatum* had negative factorial values and occurred on the negative side of the F1 axis. All variables corresponding to *H. dihystra* and *P. minor* had positive factorial values and occurred on the positive side of the F1 axis. The clumping together on one side of the F1 axis of the points corresponding to the five transects for *P. zae*, *H. dihystra*, *X. elongatum*, *P. minor* and *N. brevistilus* means that the distribution of these species was more-or-less uniform in all five transects (Fig. 12). Points corresponding to the five transects for *S. brachyurus* and *Criconemella* spp. were scattered over two or more quadrants. This was due to the fact that the distribution of these species was not the same along the five transects.

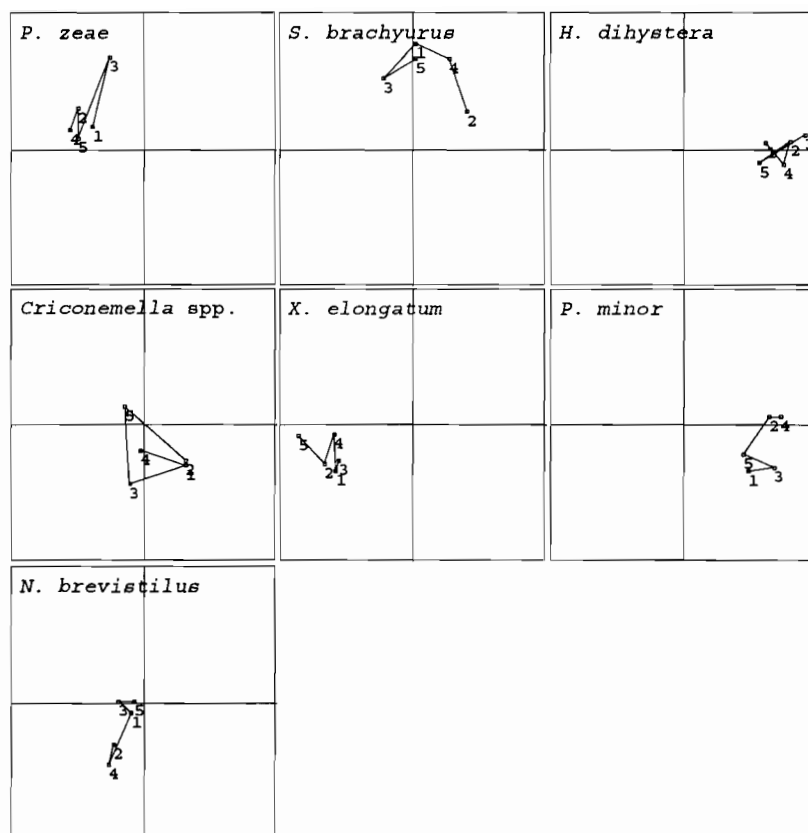


Figure 12: Projection of the five transects per nematode species on the factorial plan. (1=1st transect, 2= 2nd transect, 3= 3rd transect, 4= 4th transect, 5= 5th transect).

The factorial values for the 40 points corresponding to the samples collected along the five transects were projected on the map of the transects instead of the F1 X F2 factorial plan (Fig. 13). On the F1 factor map, two homogenous zones were clearly separated at the opposite ends with the upper part dominated by circles and the lower part dominated by squares. The demarcation showed similarity of values for the nematode species per sampling point in the two zones. The squares correspond to the negative factorial values and represented nematode communities dominated by *H. dihystra* and *P. minor* along the five transects (Fig. 13). The circles correspond to the positive factorial values and represented nematode communities dominated by *P. zaeae* and *X. elongatum*. There was no clear pattern in the distribution of circles and squares on the F2 factor map. The proportions of the nematode species were plotted per sampling point for the five transects to check if there was a change in nematode distribution along the transects (Fig. 14).

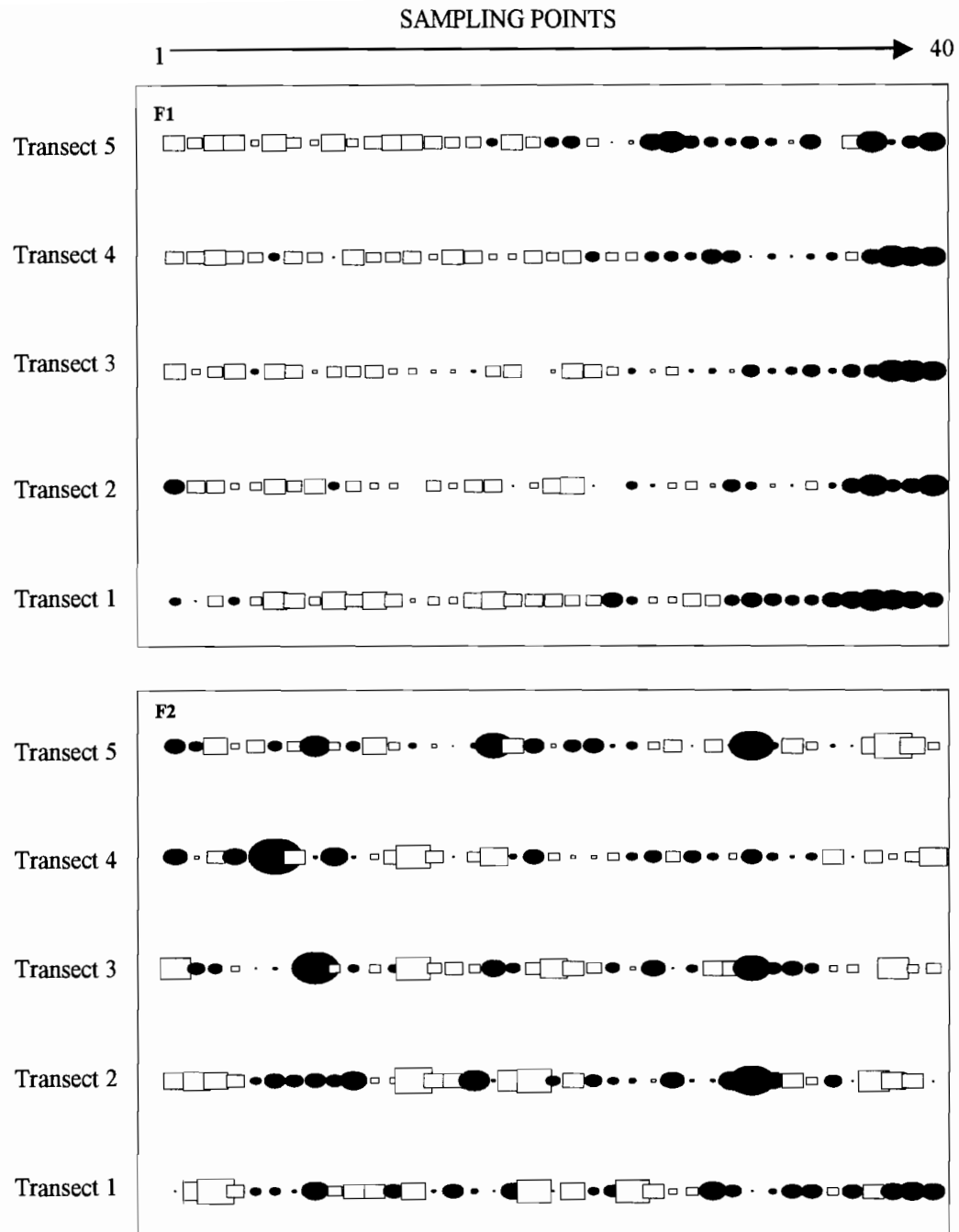


Figure 13: First and second factorial values of the PCA on the relative percentage of the nematode species projected on the trial map. Circles correspond to positive factorial values and squares correspond to negative factorial values. The size of the symbol is proportional to the absolute value.

Differences in the distribution of *X. elongatum* and *H. dihystera* were observed when their proportions were plotted along the five transects. High proportions of *X. elongatum* occurred on the first half of the transects while high proportions of *H. dihystera* occurred on the last half of the transects although this pattern was not as clear in second transect for *H. dihystera* (Fig. 14). *Paratrichodorus minor* showed a similar pattern to that of *H. dihystera*, especially in transects 4 and 5 where lower proportions occurred on the first half and higher proportions on the second half of the transects. *Pratylenchus zae* occurred in low proportions on the last half of the transect.

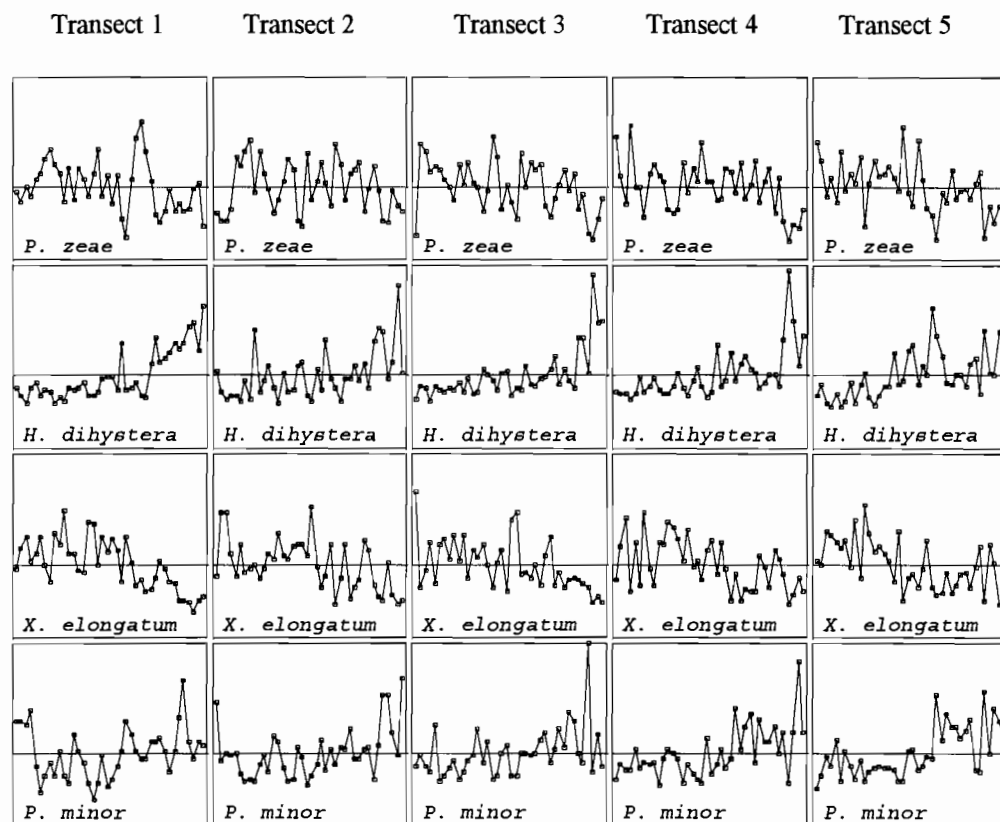
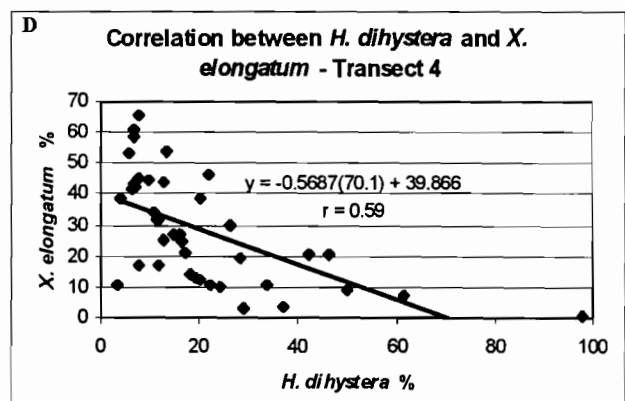
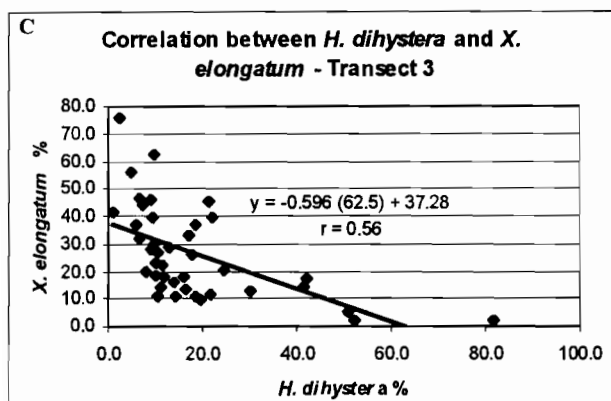
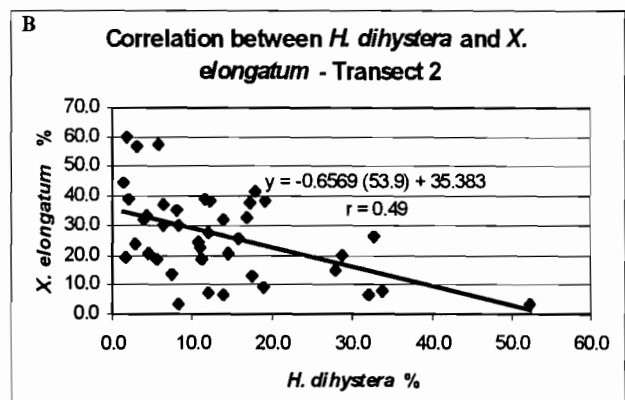
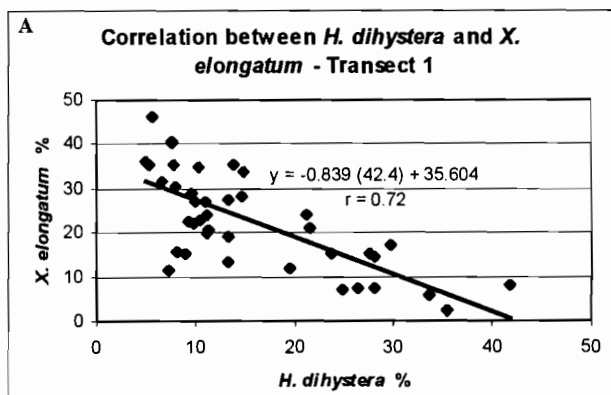


Figure 14: Changes in the relative percentages of *P. zae*, *H. dihystera*, *X. elongatum* and *P. minor* along the five transects (centred and normalised nematode values). The middle line represents the average.

When the percentages of *H. dihystra* and *X. elongatum* were plotted against each other according to the individual transects, the regression coefficients (slopes) were all negative (Figs. 15 A-E). This implies that as *H. dihystra* percentages increased, *X. elongatum* percentages decreased. In all five transects, the correlation coefficients showed that the correlation between the two species was strong. In Fig. 15a, the regression line cut the y-axis at 35.6 % and the calculated x-value was at 42.4 %, in Fig. 15b the y-intercept was at 35.3 % and the x-value was at 53.9 %. In Fig. 15c the y-intercept was at 37.3 % and the x-value at 62.5 %, in Fig. 15d the regression line cut the y-axis at 39.8 % and the x-value was at 70.1 % while in Fig. 15e the y-intercept was at 43 % and the x-value at 53.8 %. Theoretically, *X. elongatum* percentage was around 40 % when there was 0 % *H. dihystra* in each transect. *Helicotylenchus dihystra* percentage ranged between 40 % and 70 % when *X. elongatum* percentage was zero.



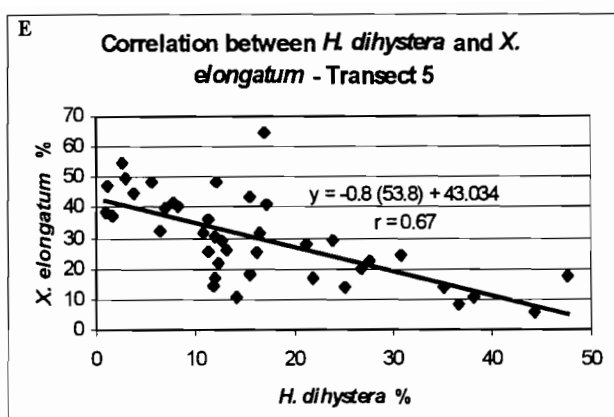


Figure 15: Significant negative regressions between *H. dihystra* and *X. elongatum* for each of the five transects with 40 sampling points each.

5.6 DISCUSSION

The average numbers of ectoparasites found per sampling point showed a clear pattern in distribution along the transect where low numbers occurred on the first half and high numbers were found on the second half. This difference in abundance may be attributed to competition or antagonism amongst the different species if occurrence of one of them in great numbers causes a decrease in others. Free-living nematodes do not depend on plants to survive and the more-or-less uniform pattern in their abundance along the transect may be due to availability of microorganisms and other environmental factors. Endoparasites depend on roots for food but their numbers in the roots showed no distinct pattern in distribution along the transect. This may be because the host plants all had the same genetic make-up and hence the food source was the same at all sampling points, although the volume of the roots would affect the nematode numbers if the growth of individual plants were different.

When the percentages of the different nematode species were plotted on the compromised factorial map, *X. elongatum* was found opposite *H. dihystra*. High percentages of *X. elongatum* occurred in places with low percentages of *H. dihystra*. When two nematode species occur within the same community, one or both species populations may be suppressed (Rao & Seshadri, 1981). Many organisms in the soil exhibit interspecific competition (Schoener, 1983) and nematodes interact with microflora and fauna in the soil. Certain microorganisms can either promote or suppress the development of certain nematode species and because the former are not uniformly distributed in the field, the latter will not be either.

The occurrence of the points representing four sampling dates close to each other and not widely distributed over the factorial maps for each species showed that there was little change in nematode communities during the crop cycle. If the nematode communities remain more or less the same over the

crop cycle, the growth period probably has little effect on these communities. However, the demarcation of the two relatively homogenous zones at the extreme ends of the transect showed that there was a difference in the distribution of nematode species. The projection of nematode proportions along the transect showed a distinct trend in which high proportions of *X. elongatum* dominated the first half of the transect and high proportions of *H. dihystra* dominated the last half. Scatter plots for the four sampling dates show that the two species do not occur in same proportions within a single sampling point.

The relative percentages of *H. dihystra* and *X. elongatum* remained stable at the sampling points over time, i.e. during the crop cycle, but were not always the same along the transect i.e. in space. The host plants had the same genetic make-up and growth rates and so the food source was the same throughout the row. If the host plant was not responsible for the observed difference in spatial distribution of nematodes, then either biotic or abiotic factors in the environment were responsible, e.g. microfauna or microflora and/or soil characteristics. Certain microorganisms are antagonistic to plant parasitic nematodes while others have a symbiotic relationship with them. Some nematode species have some affinity for certain soil elements as was shown in a large scale survey in KwaZulu-Natal where high numbers of *P. zeae*, *H. dihystra* and *P. minor* were inversely related to pH but not to Fe (Spaull & Cadet, 2001). In the same study, *Meloidogyne* spp. was inversely related to Fe but positively related to pH.

In the study of nematode spatial distribution along the five transects, the numbers of ectoparasites, free-living nematodes and endoparasites found per sampling point showed that they did not differ much in their distribution along the transects. The average densities of these nematodes remained more-or-less the same at each sampling point.

When the percentages of the different nematode species along the five transects were plotted on the compromised factorial map, *X. elongatum* was opposite to *H. dihystra*. High percentages of *X. elongatum* occurred in areas with low proportions of *H. dihystra*. As noted earlier, nematodes interact with other microorganisms that occur in the rhizosphere (Ingham, 1988). Some of these microorganisms are antagonistic to nematodes, viz. *Pasteuria penetrans* (Davies et al., 1991) while others have a positive relationship with them. Mycorrhizae forage for phosphorus and make it available to plants (Persad-Chinnery et al., 1992) and if certain nematode species have high affinity for phosphorus in the soil, that may explain all or some of the observed differences in nematode species distribution in the field.

The aggregation of the five transects either on the negative side of F1 axis for *P. zeae*, *X. elongatum* and *N. brevistilus* and on the positive side for *H. dihystra* and *P. minor* shows that there was little change in nematode communities amongst the five transects. These communities were stable amongst the transects and the spatial distribution observed on the first transect was not a chance occurrence. For *S. brachyurus*, *Criconemella* spp., *P. minor* and *N. brevistilus*, the scattering of the points corresponding to the five

transects over both positive and negative quadrants means that the distribution of these species was not the same over the five transects.

The separation of the two relatively homogenous zones at either end of the five transects proved that there were differences in nematode distribution along each transect although this pattern was the same amongst the transects. The projection of nematode species percentages along the five transects showed a clear trend in which high percentages of *X. elongatum* dominated the first halves of the transects and *H. dihyssytera* dominated the second halves of the transects. The scatter plots for the five transects showed that the two species do not occur in same percentages within any sampling point.

If the properties of *X. elongatum* and *H. dihyssytera* differ in their spatial distribution amongst the sampling point along a homogenous sugarcane row but remain the same in corresponding sampling points in adjacent rows, then the host plant is unlikely to be responsible for the observed difference. The conclusion is that the distribution of nematodes remains stable during the crop cycle and spatially along the field.

CHAPTER 6

RELATIONSHIP BETWEEN NEMATODES AND ABIOTIC SOIL FACTORS IN A SUGARCANE FIELD IN KWAZULU-NATAL

6.1 INTRODUCTION

Soil is a heterogenous medium with biological, physical and chemical properties that may vary temporally and spatially within a field (Trangmar et al., 1986). Physical soil properties can however vary between samples in an area with seemingly uniform soil (Beckett & Webster, 1971). Soil texture, or the particle size of silt, sand and clay, is another important characteristic of soils (Vaz et al., 1999). Changes in texture, mineral types and salts may determine spatial differences in soil physical properties (Wagenet & Jurinack, 1978).

Texture, or the particle size of silt, sand and clay, is an important characteristic of soils (Vaz et al., 1999). The spatial variation of soil texture governs the movement of water and solutes in soil thereby affecting root development (Berndtsson & Bahri, 1995). The availability and adsorption of phosphorus by soil in the tropics is usually influenced by clay content, Al oxides, pH and organic matter content (Agbenin & Tiessen, 1994; Bhattacharyya et al., 2003). Phosphorus is necessary for plant growth and in eliminating plant nutrient deficiencies (Needelman et al., 2001). The variability of this element in the field may also be caused by uneven distribution of fertiliser.

Soil characteristics and associated crop yields are often correlated (Timlin et al., 1998) such that spatial variability in agricultural fields results in spatially varying crop yields (Berndtsson & Bahri, 1995). In any cultivated field, it is common for crops to have uneven growth (Cadet et al., 2001) and these differences in growth can be attributed to differences in clay and water content or soil organisms. Crop yields may thus vary because of spatial heterogeneity of the biological, physical and chemical properties of the soil (Bresler & Laufer, 1988). These physical and chemical properties vary because of differences in soil material and the position of the landscape (Riha et al., 1986). Thus the spatial variability of the chemical properties in a field can influence the way the plant responds (Tsengaye & Hill, 1998) and that may lead to non-uniformity in plant growth within a field (Tsengaye & Hill, 1996).

The nematode community structure is often influenced by the chemical and physical soil parameters (Francl, 1993). A survey conducted by Spaul & Cadet (2001) showed that *Pratylenchus zeae*, *Helicotylenchus dihystra* and *Paratrichodorus minor* had a positive correlation with Fe but a negative one with pH. Soil texture can inhibit or facilitate movement of nematodes towards the food source or a mate (Norton, 1989). Some soils are unsuitable for particular nematode species and therefore contain fewer individuals or different nematode communities than more favourable environments (Cadet & Thioulouse,

1998). Nematodes can move more easily in sandy soils than in clayey soils, making it easier for them to get to food sources and mates. Differences in the texture and the nutrient properties of soil may affect the root quality, i.e., penetration rate and nutritive content. The way nematodes perceive the root exudates can be modified by changing the ion balance of the soil solution, thereby making the nematodes unable to locate their food source (Spaull & Cadet, 2001). Some soils are called resistant because the abiotic factors decrease the number of nematodes found in these soils (Amir & Alabouvette, 1993).

In chapter 5, the nematode communities found in five adjacent transects showed differences in their distribution along the transects. *Xiphinema elongatum* dominated nematode communities found on the first part of the transects while *H. dihystra* dominated communities found on the second half of the transects. The difference in the distribution of the two species could not be attributed to the host plant, sugarcane, as it was propagated vegetatively and hence all plants had the same genetic make-up and only rows that showed uniform growth by visual observation were chosen. Food volume and availability were not the cause. Abiotic factors, i.e., soil elements and soil types, were therefore studied to determine if they were also uniformly distributed along the five transects.

A. SPATIAL DISTRIBUTION OF ABIOTIC SOIL FACTORS ALONG THE TRANSECT

6.2 MATERIALS AND METHODS

See chapter 3, section 3.4.

6.3 RESULTS

The analysis of the soil elements data using Three-Ways Table Analysis showed that sampling points 26 and 27 differed in their distribution from the other sampling points when plotted on factor maps per transect. This pattern was observed in the first and second transects while only sampling point 27 was different in the third, fourth and fifth transects (Fig. 1). The soil samples collected from these sampling points had extremely high levels of magnesium, calcium and sodium.

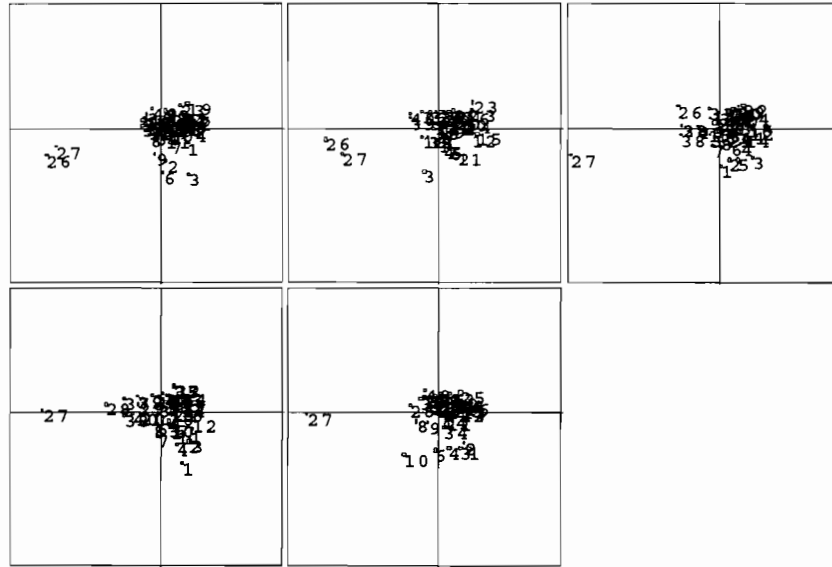


Figure 1: Row distribution according to soil element and soil type values for the 40 sampling points in each of the five transects.

The factorial values of the 40 points corresponding to the soil samples collected along the five transects were projected on the map of the transects instead of on the F1 X F2 factorial plan (Fig. 2). On the F1 factor map, the first part showed areas of circles corresponding to positive factorial values alternating with areas with squares corresponding to negative factorial values while the second part was predominantly squares. The circles and squares are variables corresponding to positive and negative factorial values of soil elements per sampling point, respectively. On the F2 factor map, the first part, the first 10 sampling points, was dominated by squares while the rows thereafter were dominated by circles although some rows had squares or negative factorial values. On the F1 factor map, rows 26 and 27 again had high negative factorial values and this was also shown on the F2 factor map, although less so. This difference showed similarity of values for the soil elements and soil particle characteristics per sampling point in the two areas, one with high proportions of *X. elongatum* and the other with high proportions of *H. dihystra*.

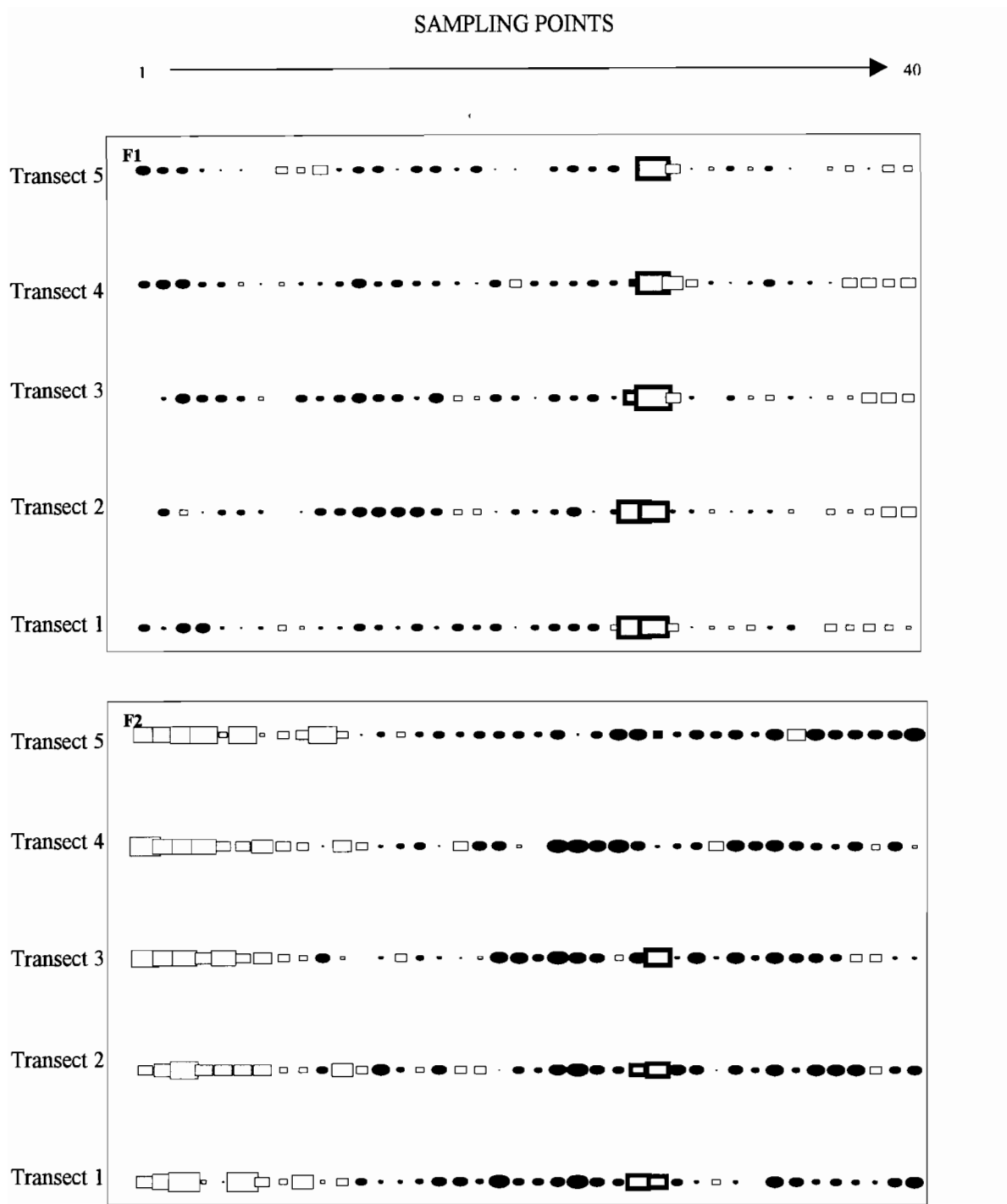


Figure 2: First and second factorial values of the PCA on the values of soil elements and soil particles for the 40 sampling points projected on the trial map. Circles correspond to positive factorial values and squares correspond to negative factorial values. The size of the symbol is proportional to the absolute value.

The compromised factor map of the soil element variables described 52.2 % and 16.3 % of the variability for the F1 and F2 axes respectively. The variables corresponding to Ca, Mg, Na, Mn, Zn, K, clay and silt had high negative F1 factorial values while variables corresponding to medium sand had high positive F1 factorial values (Fig. 3). Variables corresponding to Fe had high positive F2 factorial values while those corresponding to coarse sand had high negative F2 factorial values. Variables corresponding to Al, S, pH, P and fine sand lay at the centre of the factorial map, with very low factorial values.

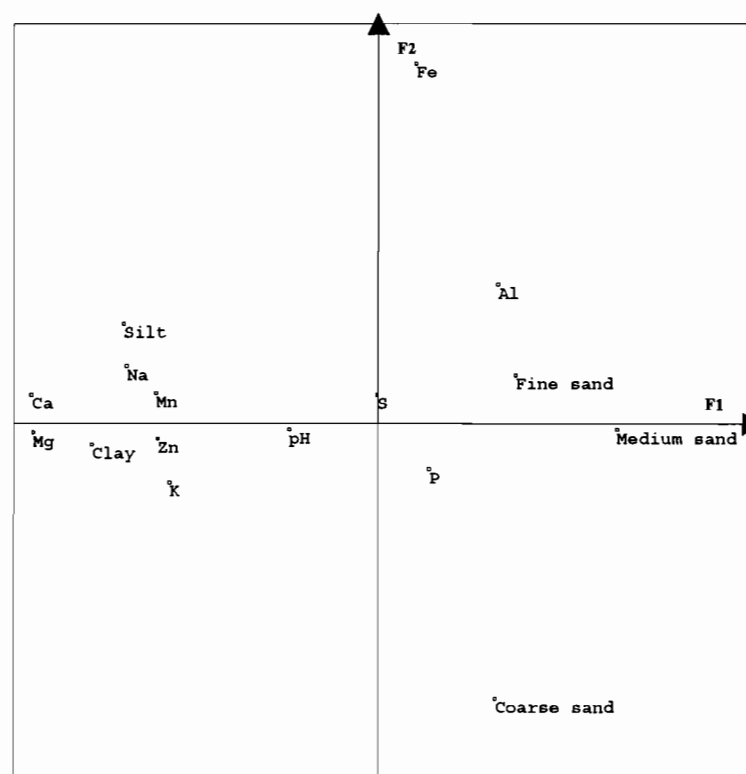


Figure 3: Compromised factorial map (F1 X F2) of the Three-Ways Table Analysis on soil element and particle variables for the 40 sampling points along the five transects.

The distribution of the individual soil elements and particles shown in Fig. 3 was also demonstrated in Fig. 4 but according to the five transects. For the five transects, the variables corresponding to pH, K, Ca, Mg, Na, Zn, Mn silt and clay occurred on the negative side of F1 while variables corresponding to Al, fine sand and medium sand occurred on the positive part of F1 and had positive factorial values. Variables corresponding to Fe occurred on the positive side of F2 while those corresponding to coarse sand occurred on the negative side of F2. These soil elements and particles did not change much amongst the five transects. Although variables corresponding to P and S were scattered along the positive and negative sides of F1, they showed some homogeneity in their distribution along the five transects (Fig. 4).

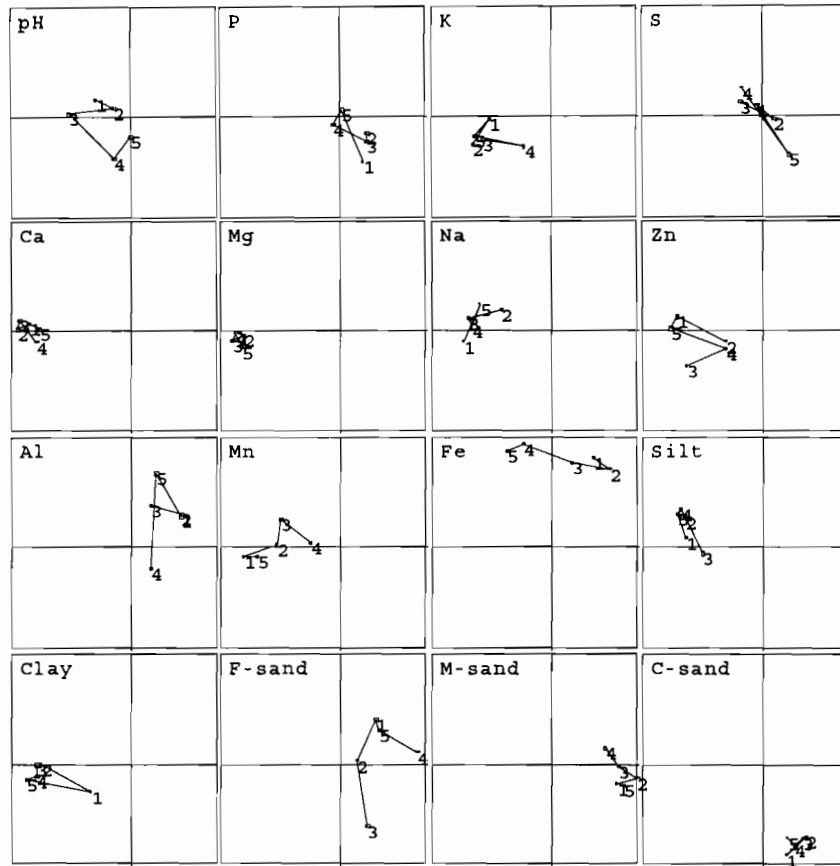
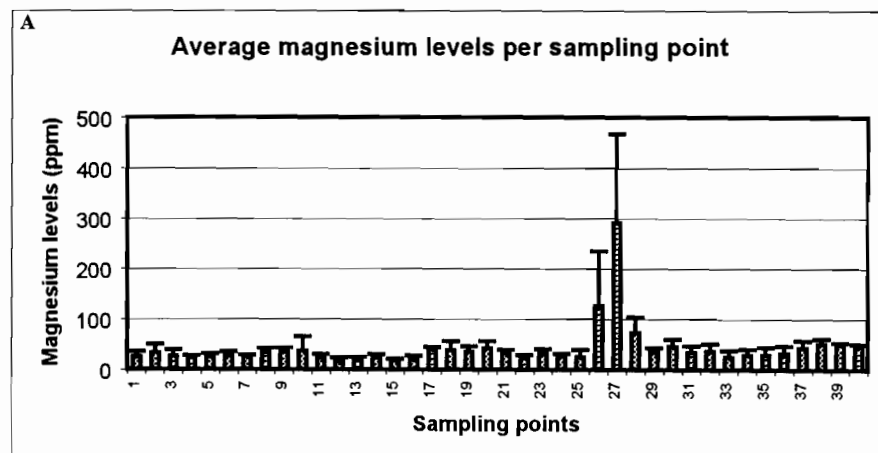
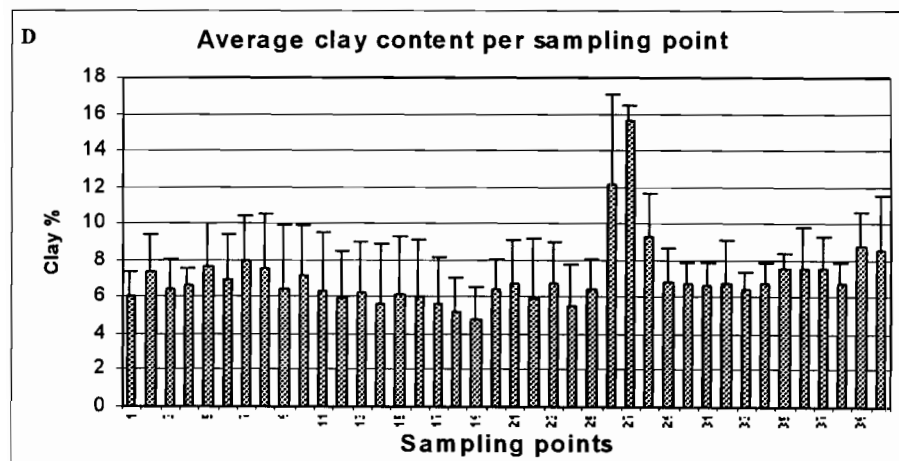
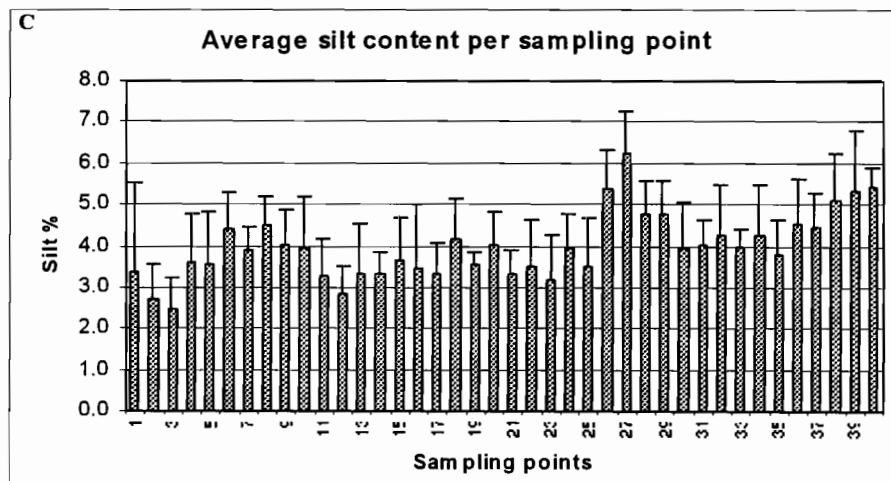
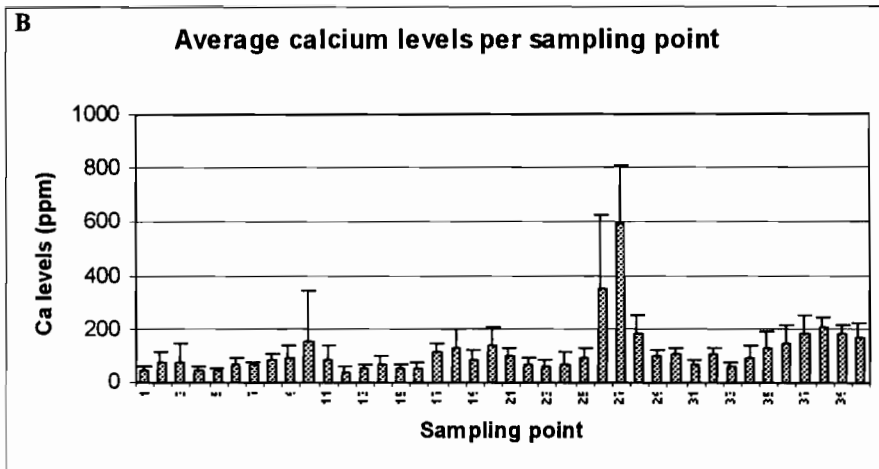


Figure 4: Projection of the five transects per soil elements and particles on the factorial plan (1 = transect 1, 2 = transect 2, 3 = transect 3, 4 = transect 4, 5 = transect 5).

Samples 26 and 27 had very high element and particle values, especially Mg, Ca, silt and clay and low values for fine and medium sand (Fig. 5 a -e). The distribution of the individual soil particles along the transects showed homogeneity for silt, clay, fine sand and medium sand while the coarse sand content was higher on the first part of the transect, gradually decreased in the middle and increased again in the last seven sampling points (Figs. 5 c-g).





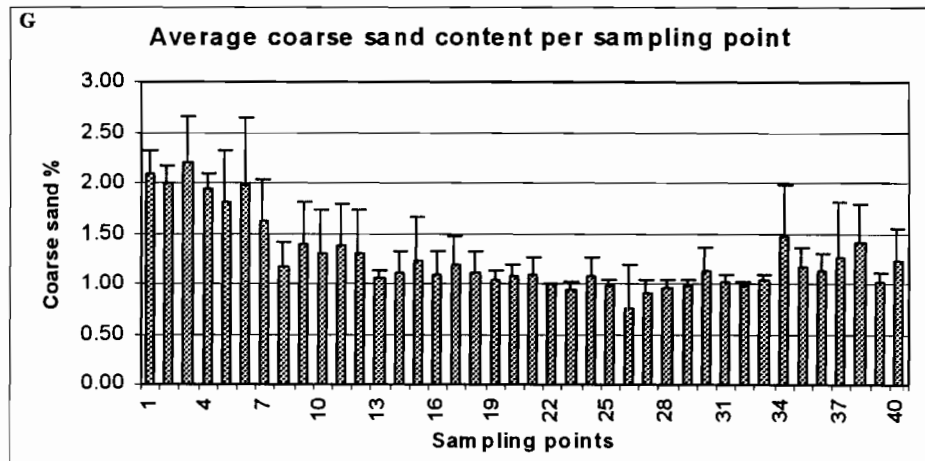
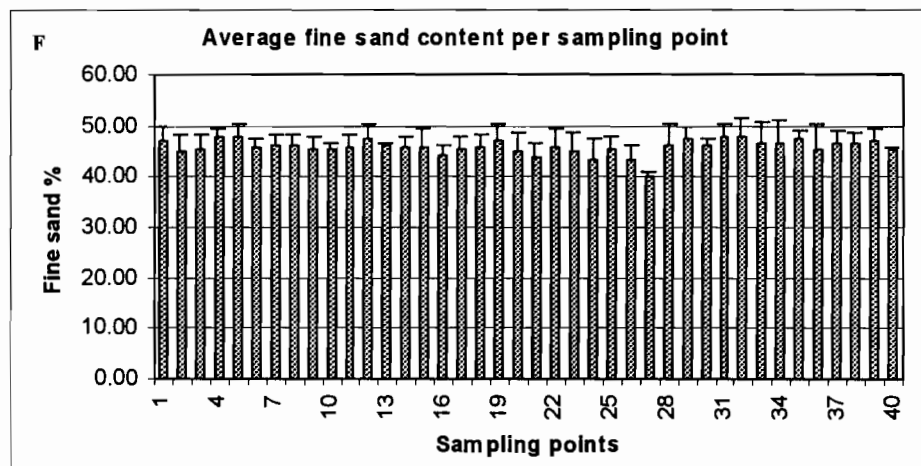
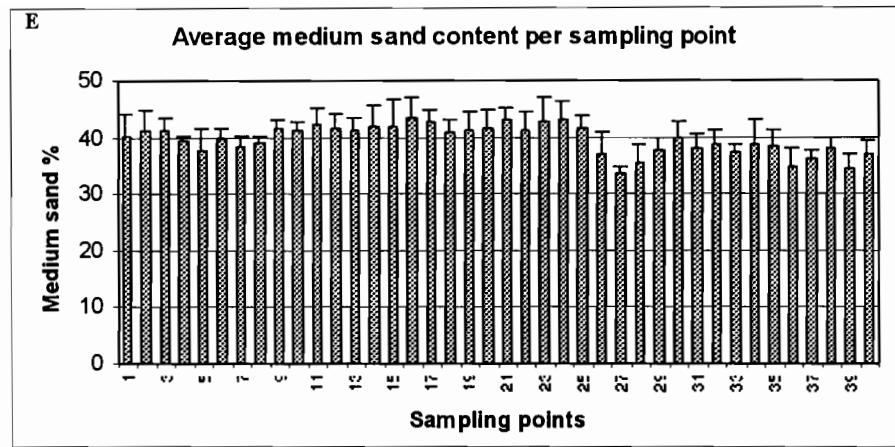
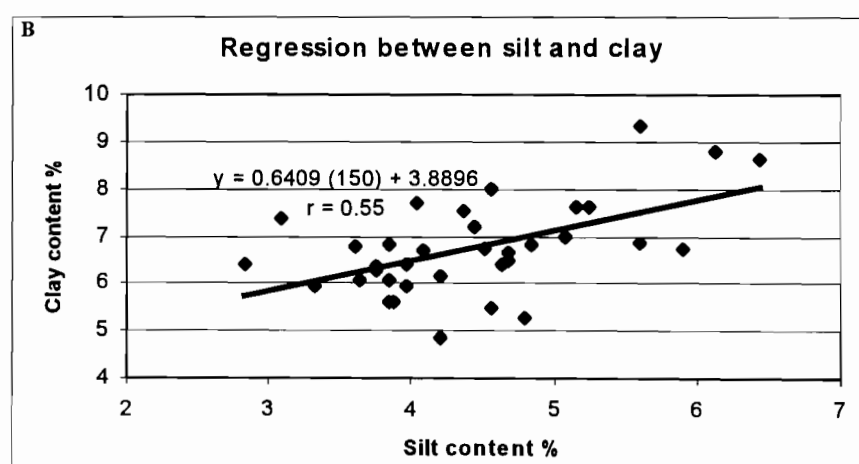
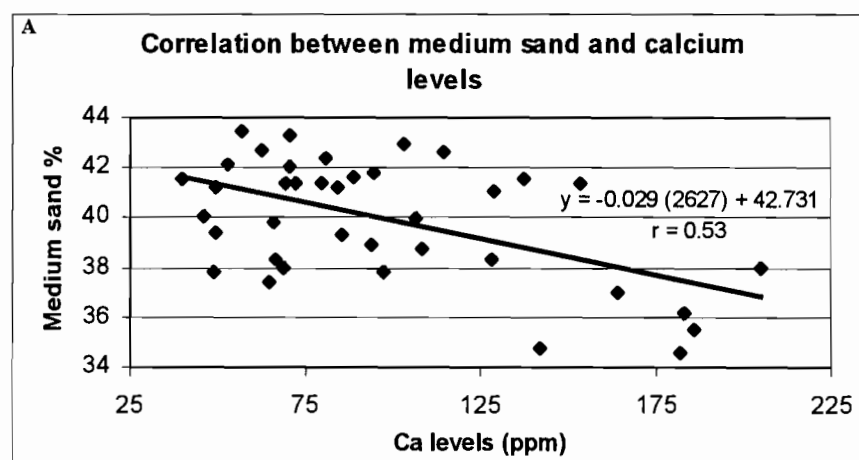


Figure 5: Distribution of magnesium (A) and calcium (B) levels, silt (C), clay (D), medium sand (E), fine sand (F) and coarse sand (G) content at the 40 sampling points along the five transects. Bars represent standard errors.

When medium sand values, which had a strong positive correlation with the first factor were plotted against calcium level values, which had a strong negative correlation with the first factor, the slope, $b = -0.029$, was negative (Fig. 6a). The correlation coefficient, $r = 0.53$ showed a strong correlation between the two variables, the regression line cut the y-axis at 42.7 % while the calculated x-intercept was at 2627 ppm. When the regression between silt and clay contents was determined, the regression line cut the y-axis at 3.8% and the x-axis at 150% (Fig. 6b). The positive correlation coefficient, $r = 0.55$, and the slope, $b = 0.64$ showed a strong correlation between the two soil types. When clay and medium sand values were plotted against each other, the slope, $b = 0.28$ was negative and the correlation coefficient, $r = 0.73$ implied a strong and negative correlation between the two soil types (Fig. 6c). The y-intercept was at 18.3% while the x-intercept was at 63.2 %. In Fig. 6d, the regression coefficient, the slope = 0.12 between magnesium and aluminium was negative. The correlation coefficient, $r = 0.35$ showed a strong correlation between the two variables. The y-intercept was at 21.4 ppm while the x-intercept was at 166 ppm.



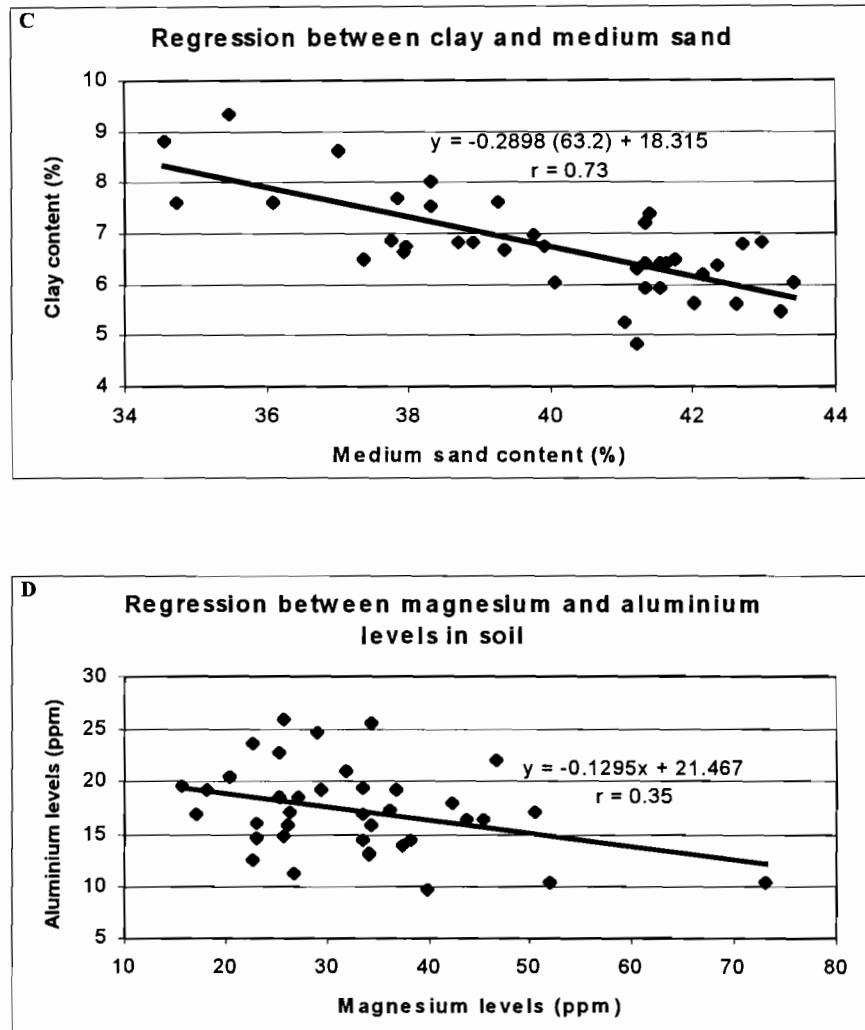


Figure 6: Negative regressions between medium sand and calcium levels (A), positive regressions silt and clay (B), a negative regression between clay and medium sand (C) and magnesium and aluminium (D) at the 40 sampling points along the five transects.

B. NEMATODE DISTRIBUTION IN RELATION TO ABIOTIC SOIL FACTORS

In chapter 5 , the nematode communities showed differences in their distribution along the transects, with *X. elongatum* dominating on the first part of the transects while *H. dihystra* dominated on the second half of the transects. In the soil study, Ca and Mg occurred at high levels in areas with high silt and clay content and at low levels in areas with high fine, medium and coarse sand content. Environmental factors have an important effect on nematode communities since nematodes spend part or all of their lives in the soil.

Abiotic factors, i.e. soil elements, were therefore explored to determine if they were responsible for the observed species distribution patterns.

6.4 MATERIALS AND METHODS

See chapter 3, section 3.5.

6.5 RESULTS

Both nematode and soil data were first analysed using Principal Component Analysis (PCA). The aim of this is to find an axis along which the cloud of points is the longest (Thioulouse et al., 1997), the length of this axis corresponding to the importance of a factor. PCA also highlights correlation between variables and emphasizes the existence of ecologically-defined groups of individuals.

Rows 26 and 27, corresponding to sampling points 26 and 27 in the field, were removed from both nematode and soil data. Principal Component Analysis performed on the nematode data with 38 rows showed that the factorial map described 46.23 % and 21.3 % of the variability for F1 and F2, respectively. The correlation circle showed that the determinant variables for *P. zae* and *X. elongatum* correlated with the positive value of F1 and were opposite *H. dihystra* and *P. minor* which correlated with the negative value of F1 (Fig. 7a). The variables for *S. brachyurus* correlated with the positive value of F2 while variables for *Criconemella* spp. and *N. brevistilus* correlated with the negative value.

The PCA performed on soil data with 38 rows showed that the factorial map described 33.61 and 17.53 % of the variability for F1 and F2 respectively. The variable for medium sand correlated with the positive value of F1 while the variables for silt, calcium, magnesium, manganese, clay and zinc correlated with the negative value (Fig. 7b). No relationship between nematode species and soil characteristics could be extracted from the PCA factor maps as PCA only highlights a correlation between variables with emphasis on groups of individuals from the same data. The analysis of the nematode data was done independently of the soil characteristics data and hence no correlation between the variables from the two datasets could be extrapolated.

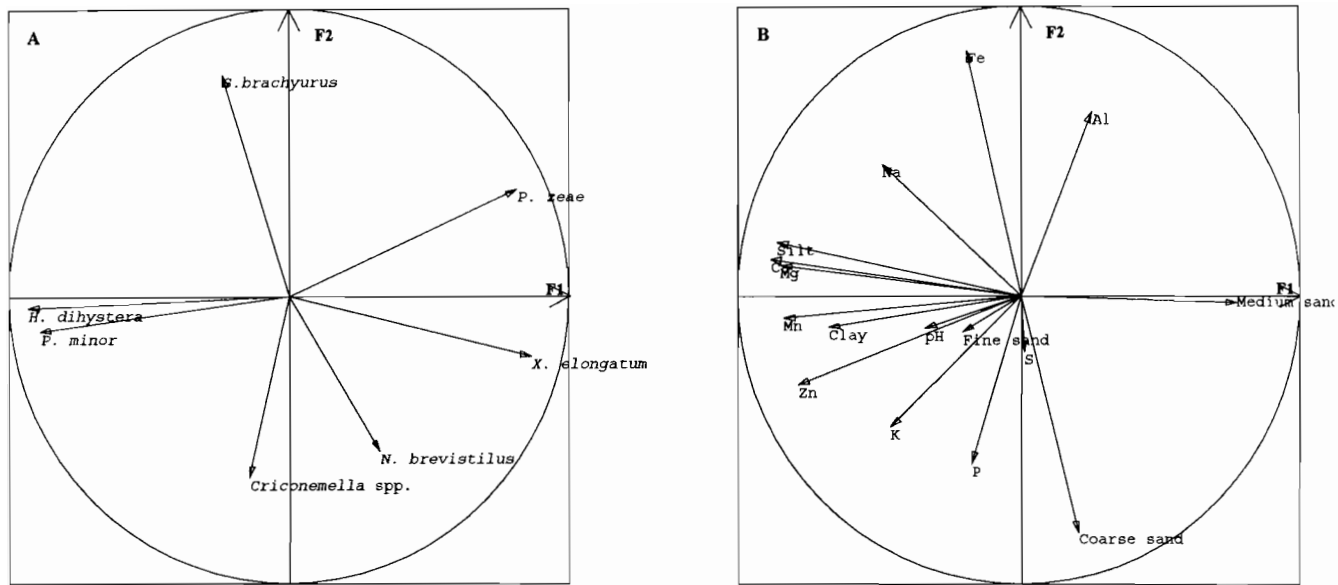


Figure 7: Correlation circles (F1 X F2) of the PCA on nematode species (A) and soil element and soil particle data (B) for the 38 sampling points (average) along the five transects.

Prior to coinertia analysis, a permutation test was computed to show whether or not the relationship between two tables is significant. This test showed that the relationship was statistically significant, i.e. soil elements and particles were related to nematode species, either positively or negatively ($p < 0.000$) (Fig. 8). The rows from the two datasets were randomly matched a 1000 times but the relationship between them remained statistically significant and the observed value is thus valid over the set of random permutations.

number of random matching: 1000 Observed: 2.949108
Histogram: minimum = 0.172478, maximum = 2.949108
number of simulation X<Obs: 1000 (frequency: 1.000000)
number of simulation X>=Obs: 0 (frequency: 0.000000)

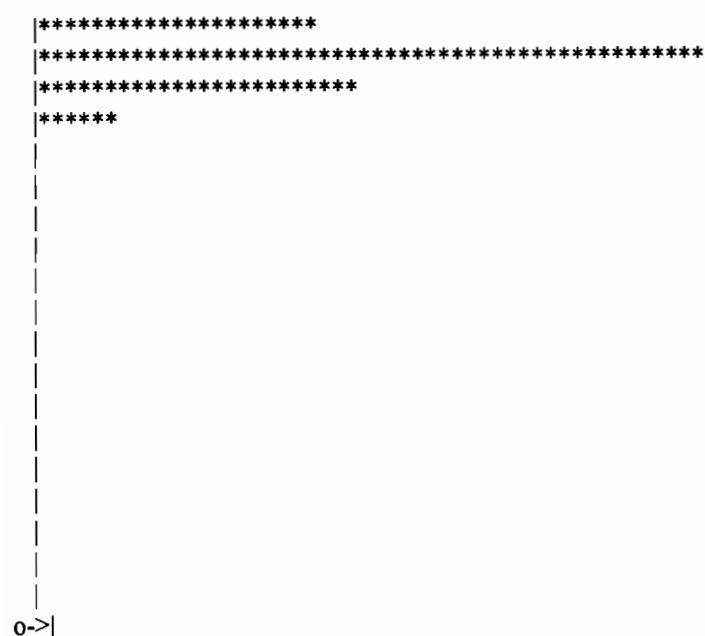


Figure 8: Permutation test of the coinertia analysis for the nematode species and soil element data in the 38 sampling points.

Because the permutation test was significant, the coinertia analysis was performed. The coinertia factor maps are drawn from two datasets and highlight a link amongst the variables from the two sets of data. They differ from PCA factor maps because they show a correlation between variables from two independent datasets, unlike PCA factor maps which show a correlation amongst variables from the same set of data. The coinertia factor map resulting from the analysis of the nematological data showed that the variables corresponding to *H. dihystra* and *P. minor* had high negative F1 factorial values while the variables corresponding to *P. zae* and *X. elongatum* had high positive factorial values along this axis (Fig. 9a). The variables corresponding to *S. brachyurus* had positive factorial values along the F2 while variables corresponding to *N. brevistilus* and *Criconebella* spp. had high negative F2 values. The soil element coinertia map showed that the determinant variables corresponding to Mn, Mg, Na, Ca, clay and silt had high negative F1 factorial values while variables corresponding to medium sand had high positive F1 factorial values. Variables corresponding to Fe had high negative F2 values. Variables corresponding to K, Zn, Al, pH, P, S, fine and coarse sand had low factorial values and lay almost at the centre (Fig. 9b).

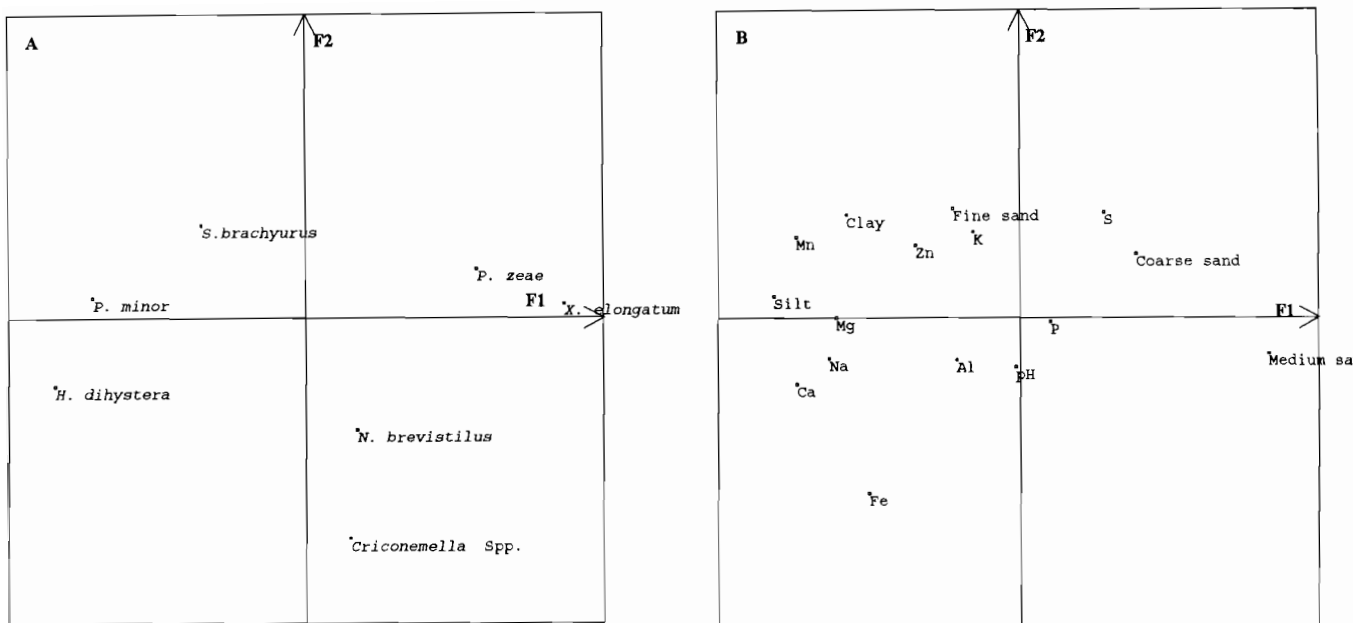


Figure 9: Factor maps (F1 X F2) of the coinertia analysis on nematode species (A) and soil element data (B) in the 38 rows along the five transects.

The correlation between nematodes and soil elements and particles shown in Fig. 3 was summarised by a crossed table for the two datasets (Fig. 10). The circles show positive correlation while the squares show negative correlation. *Helicotylenchus dihystra* and *P. minor* had positive correlation with almost all the soil elements and particles except S, medium and coarse sand and also P for *P. minor*. *Xiphinema elongatum* and *P. zae* had negative correlations with almost all the elements and particles except pH, P, S, medium sand and coarse sand although *P. zae* had a negative correlation with pH as well. *Scutellonema brachyurus* had positive correlations with almost all the soil elements and particles except P, Na, medium and coarse sand. *Criconemella* spp. had a negative correlation with all the soil elements and particles except pH, Al, Mn, Fe and medium sand. *Neodolichodorus brevistilus* had a negative correlation with all except Mg and medium sand.

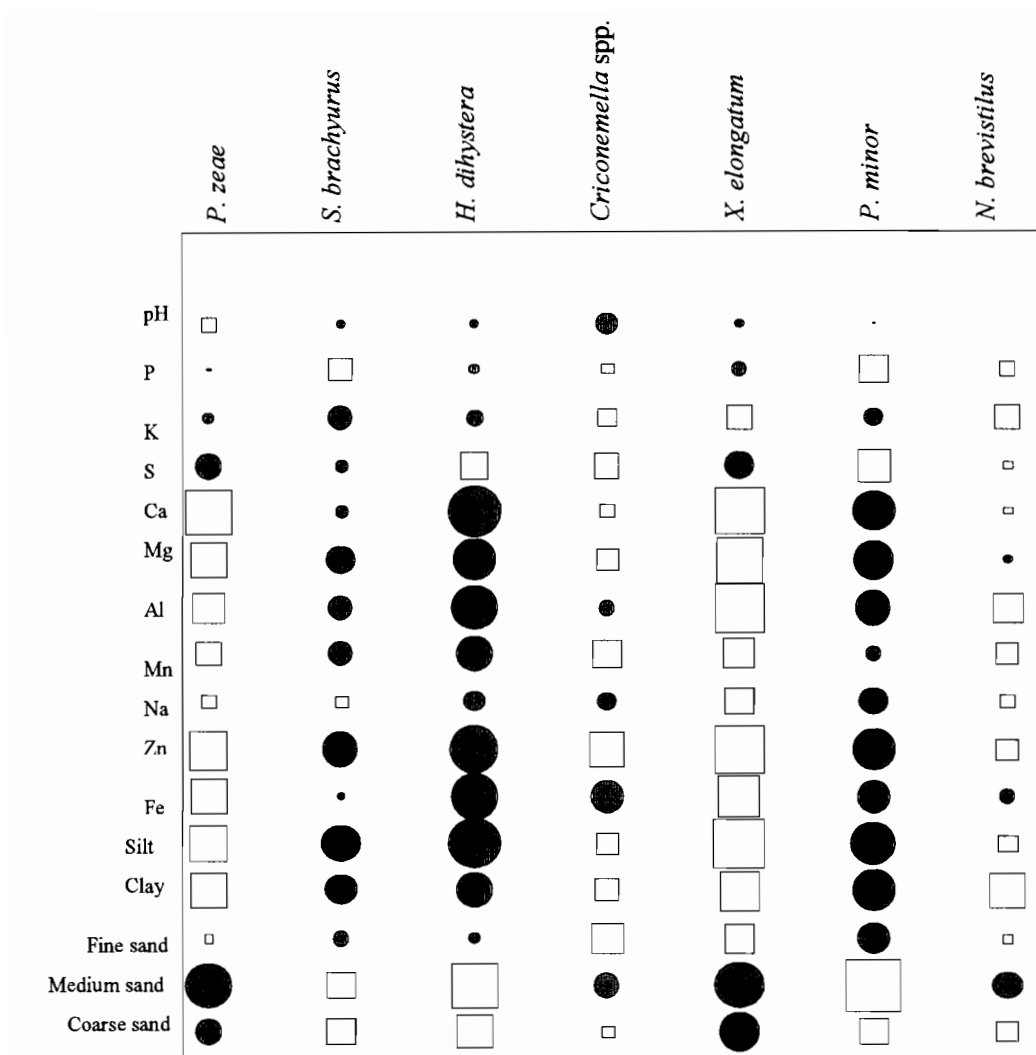


Figure 10: Crossed coinertia table showing the relationship between nematode species and soil elements. Circles represent a positive correlation while squares represent a negative correlation. The size of the symbol is proportional to the absolute value.

When scatter plots of *H. dihystra* against magnesium (a) *X. elongatum* against magnesium (b) and medium sand against *H. dihystra* (c) were drawn, the regression was negative for the first one and negative for the last two (Fig. 11). The correlation coefficient, $r = 0.52$ for *H. dihystra* and magnesium showed that the two variables had a strong correlation between them. The positive regression coefficient, slope = 0.47, implied that as magnesium levels increased, *H. dihystra* percentages increased (Fig. 11a). The regression line cut the y-axis at 1.58 % while the x-value was at 210 ppm. The correlation between *X. elongatum* and magnesium had a negative slope implying that *X. elongatum* percentages decreased with an increase in magnesium level (ppm) (Fig. 11b). The correlation coefficient, $r = 0.56$ showed that the correlation

between the two variables was strong. The y-intercept was at 43.5 % while the calculated x-value was at 88 ppm.

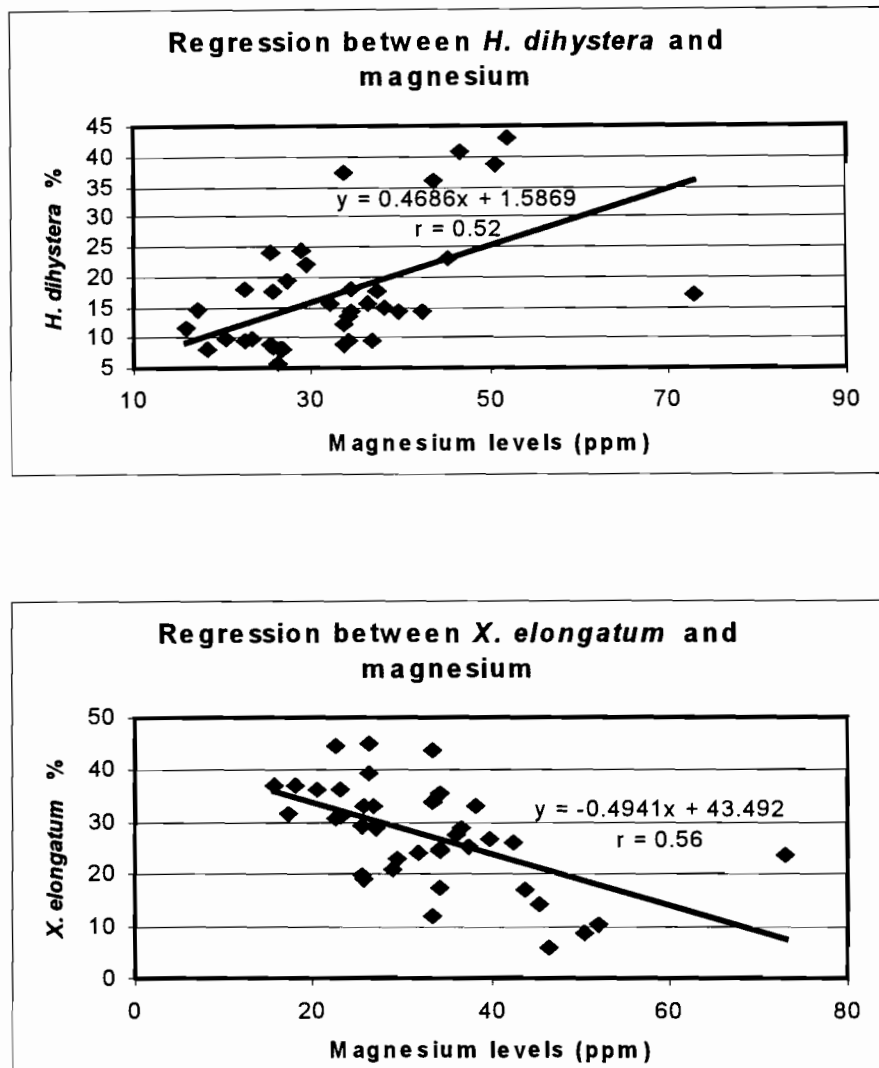


Figure 11: Scatter plots showing a positive correlation between *H. dihystra* and magnesium (A) and a negative correlation between *X. elongatum* and magnesium (B).

6.6 DISCUSSION

Changes in soil texture, mineral content and salts may determine spatial differences in the physical properties of soils (Wagenet & Jurinack, 1978). This was shown in this study by the high levels of Mg and Ca, the high silt and clay content and low medium sand content at sampling points 26 and 27 from which soil samples were taken along the five transects. The reason may be due to a water runaway that may pass through this part of the field carrying with it soil nutrients and elements from elsewhere.

The occurrence of Ca and Mg on the same side of the F1 axis as silt and clay shows that these elements have a positive correlation with these soil types while they have an inverse relationship with the coarser sands. Calcium and Mg were inversely correlated with P as these elements occurred on the negative and positive sides of the F1 axis respectively. The heterogeneity in soil texture is well shown as silt and clay soils occurred together along the first axis but not in areas where fine and medium were found. This strong particle size gradient implies that silt and clay were less common in areas with high fine and medium sand contents. Silt, clay, fine and medium sand particles showed a uniform distribution along the transects, except coarse sand. High coarse sand content occurred on the first part of the transects, decreasing gradually in the middle and increased again over the last sampling points.

The soil elements also showed heterogeneity in their occurrence along the five transects, with high P and Al levels occurring at sampling points with low Ca, Mg, Na, Mn, Zn and K levels. The correlation of Fe with the other soil elements was weak as it occurred along the second axis. In Fig. 6a, the negative correlation showed that high calcium levels were found in sampling points with low medium sand content. In Fig. 6 b, clay content increased as silt content increased. In Fig. 6c, the negative correlation between clay and medium sand implied that as clay content increased, medium sand content decreased and vice versa. The y-intercept theoretically meant that sampling points with 18% of clay had 0% of medium sand while the x-intercept meant that sampling points with 63% medium sand had 0% clay. In Fig. 6d, the negative correlation between magnesium and aluminium implied that levels of magnesium in soil decreased as levels of aluminium increased. The value of the y-intercept meant, theoretically, that sampling points with 21 ppm of aluminium had no magnesium. Also, the x-intercept meant that sampling points with 166 ppm magnesium had no aluminium.

In the section relating nematodes to abiotic soil factors, the nematode species PCA showed that *H. dihystra* and *P. minor* were on opposite sides to *P. zae* and *X. elongatum* along the first factor which identifies the main differences. This may be attributed to differences in proportions within their communities, where higher proportions of the first two species may lead to lower proportions of the latter two species. Certain biotic and abiotic factors which favour the establishment and expansion of some of

these nematode species may also play a role in determining the way nematodes are distributed along the length of the field.

The scatter plot drawn between *H. dihystra* and magnesium had a positive correlation implying that *H. dihystra* percentages increased with an increase in soil magnesium levels (Fig. 11a). Theoretically, 100 % *H. dihystra* could be found in areas with 210 ppm levels of magnesium. The negative correlation between *X. elongatum* and magnesium supposedly showed that 43.5 % of *X. elongatum* was found in areas with 0 ppm magnesium while in areas with 88 ppm magnesium, no *X. elongatum* was found (Fig. 11b). The scatter plot drawn between medium sand and *H. dihystra* was negative implying that as medium sand content increased, *H. dihystra* percentage decreased and vice versa.

The distribution of the soil abiotic factors was uniform along the five transects and had an influence on the distribution of nematode species as high percentages of *H. dihystra* were found in silt or clay soils with high levels of magnesium while the reverse was true for *X. elongatum*.

CHAPTER 7

RELATIONSHIP BETWEEN NEMATODES AND LEAF ELEMENTS IN A SUGARCANE FIELD IN KWAZULU-NATAL

7.1 INTRODUCTION

The leaves of a plant play a major role in yield production as photosynthesis takes place in them. Young plant development controls leaf formation (Awal & Ikeda, 2003). Faster vegetative development allows the leaves to intercept more light for photosynthesis.

The uptake of Zn by plants is a continuous process that leads to Zn depletion in the root zone (Rupa et al., 2000). An increase in Zn concentration in the soil has been shown to result in P deficiency in beans (Ruano, 1987) and in tomatoes (Kaya et al., 2000). However, low Zn in the roots may lead to P toxicity in plants causing an increase in P levels in the leaves. Soil pH determines the metal sorption-desorption in soils because hydrogen ions affect the surface's ability to attract positively charged cations. Soil pH also influences P solubility and uptake. Low solubility of inorganic P in soils leads to inadequate plant nutrition.

From the previous studies conducted (Chapter 5), nematode community distribution was found to be more-or-less the same spatially amongst the five transects along which they were sampled. The distribution of soil elements and particles along the five transects also remained uniform per sampling point amongst the five transects (Chapter 6). Leaf elements were therefore examined at to check if their levels remained stable in the leaves of plants along the five transects.

A. SPATIAL DISTRIBUTION OF LEAF ELEMENTS IN A SUGARCANE FIELD.

7.2 MATERIALS AND METHODS

See chapter 3, section 3.6.

7.3 RESULTS

The leaf data analysis using Three-Ways Table Analysis showed that the first sampling point differed in its distribution from the other sampling points when plotted on a factor map. This was observed in the fourth transect (Fig. 1b). The leaf samples collected from this sampling point showed extremely high values of nitrogen and manganese.

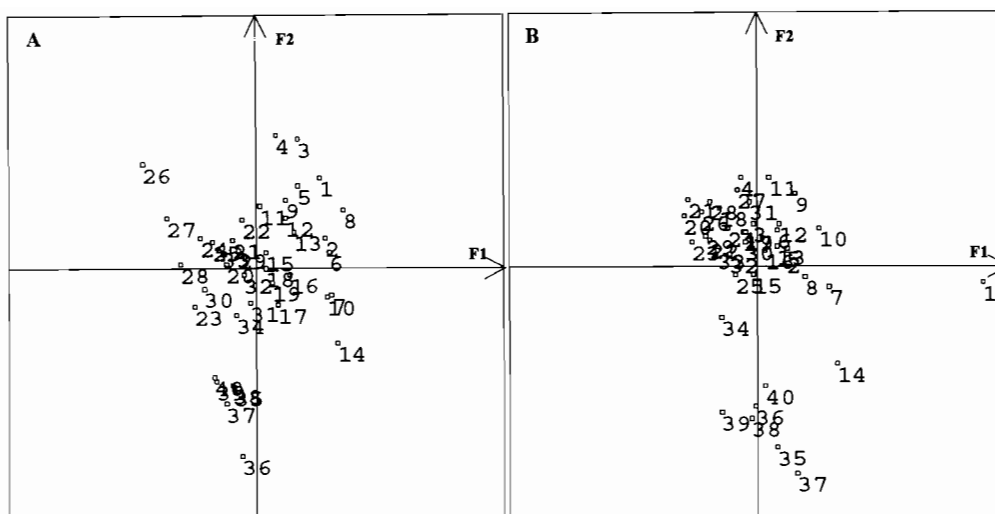


Figure 1: Factor map showing distribution of the 40 leaf sampling points in relation to the values of elements found per leaf sample in transects 1 (A) and 4 (B).

The factorial values of the 40 points corresponding to the leaf samples collected along the two transects were projected on the map of the transects instead of on the F1 X F2 factorial plan (Fig. 2). On the F1 factor map, the first part showed areas of circles corresponding to positive factorial values while the second part was dominated by squares corresponding to negative factorial values. On the F1 factor map, row 1 in transect four showed high positive factorial values. On the F2 factor map, the first part was dominated by circles interspersed by squares while the seven last rows were dominated by squares. This difference in areas dominated by circles and squares showed similarity of values for the leaf element characteristics per sampling point in the two halves of the transects with different species proportions.

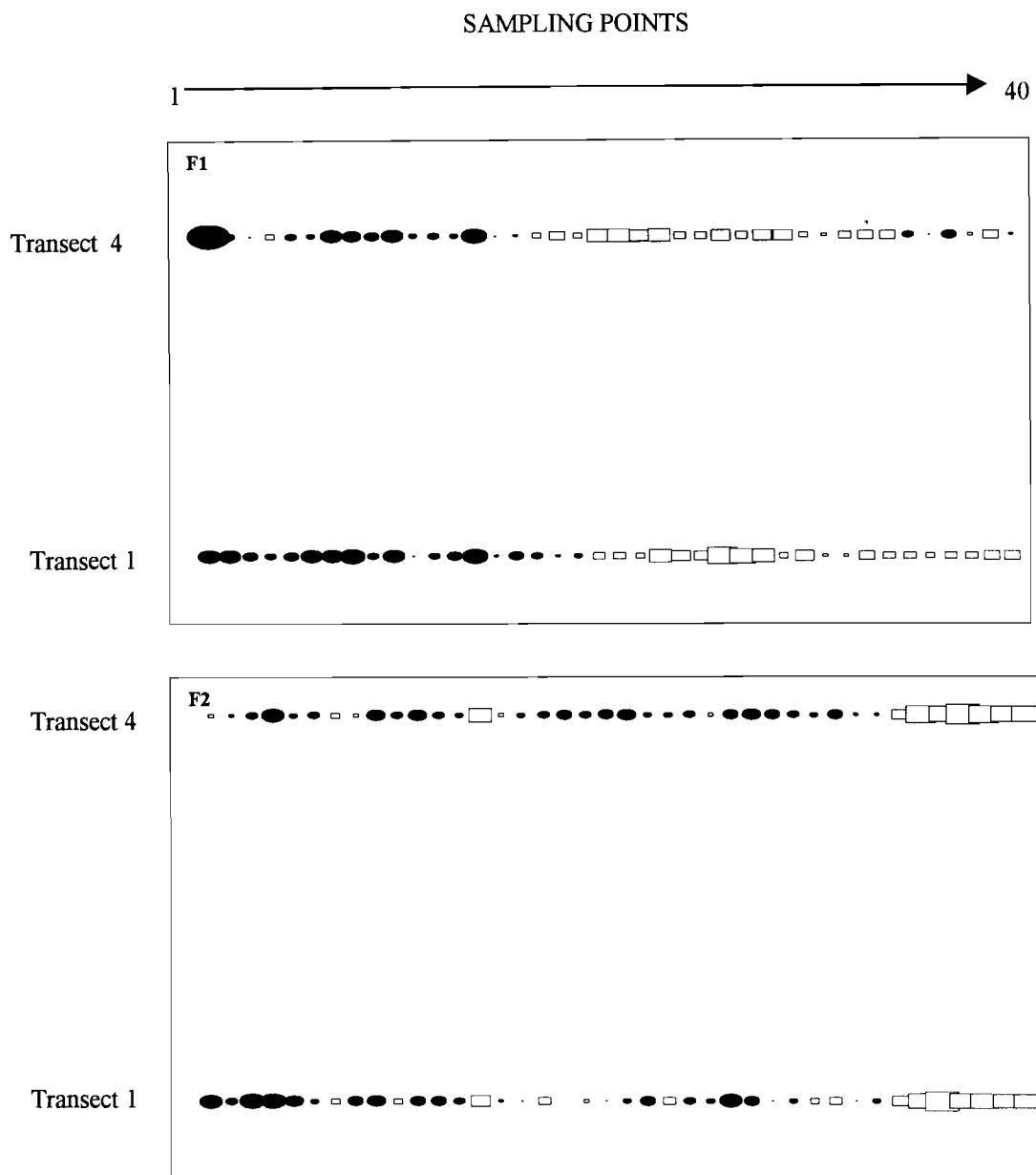


Figure 2: First and second factorial values of the PCA on the values of soil elements and soil particles projected on the trial map. Circles correspond to positive factorial values and squares correspond to negative factorial values. The size of the symbol is proportional to the absolute value.

The PCA performed on the leaf data with 80 rows showed that the factorial map described 33.7 % and 21.4 % of the variability for F1 and F2 respectively. The variables corresponding to P, K and Cu had a positive association amongst themselves and were correlated with the positive part of the first factor (Fig. 3).

Variables corresponding to Mg, Zn and N had a positive association amongst themselves and were also correlated with the positive part of F1. Variables corresponding to Ca and Fe had an association between themselves and were correlated with the negative part of F2 while S and Mn also had an association between them and also correlated with the F2.

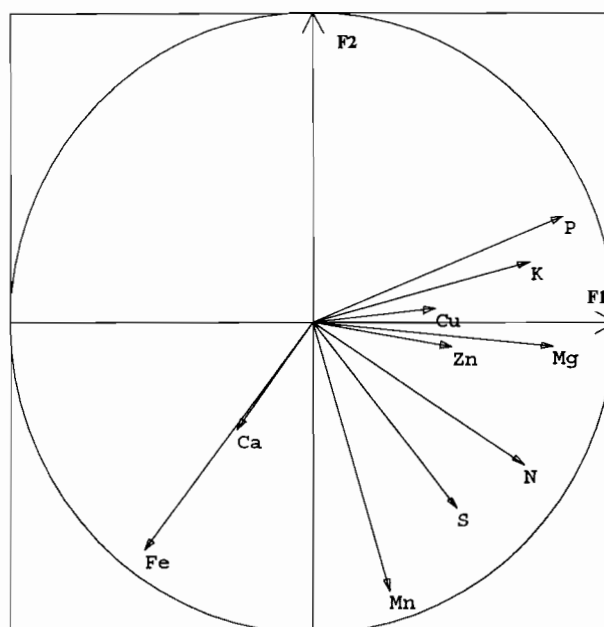


Figure 3: Correlation circle (F1 X F2) of the PCA on leaf element data from 40 sampling points in transects 1 and 4.

The original data showed that the first sampling point in transect 4 had high leaf element values, especially N while the last six sampling points had high calcium levels (Figs. 4a & b). The two homogenous zones with circles and squares showed that areas dominated by circles had high N levels while those dominated by squares had high levels of Ca.

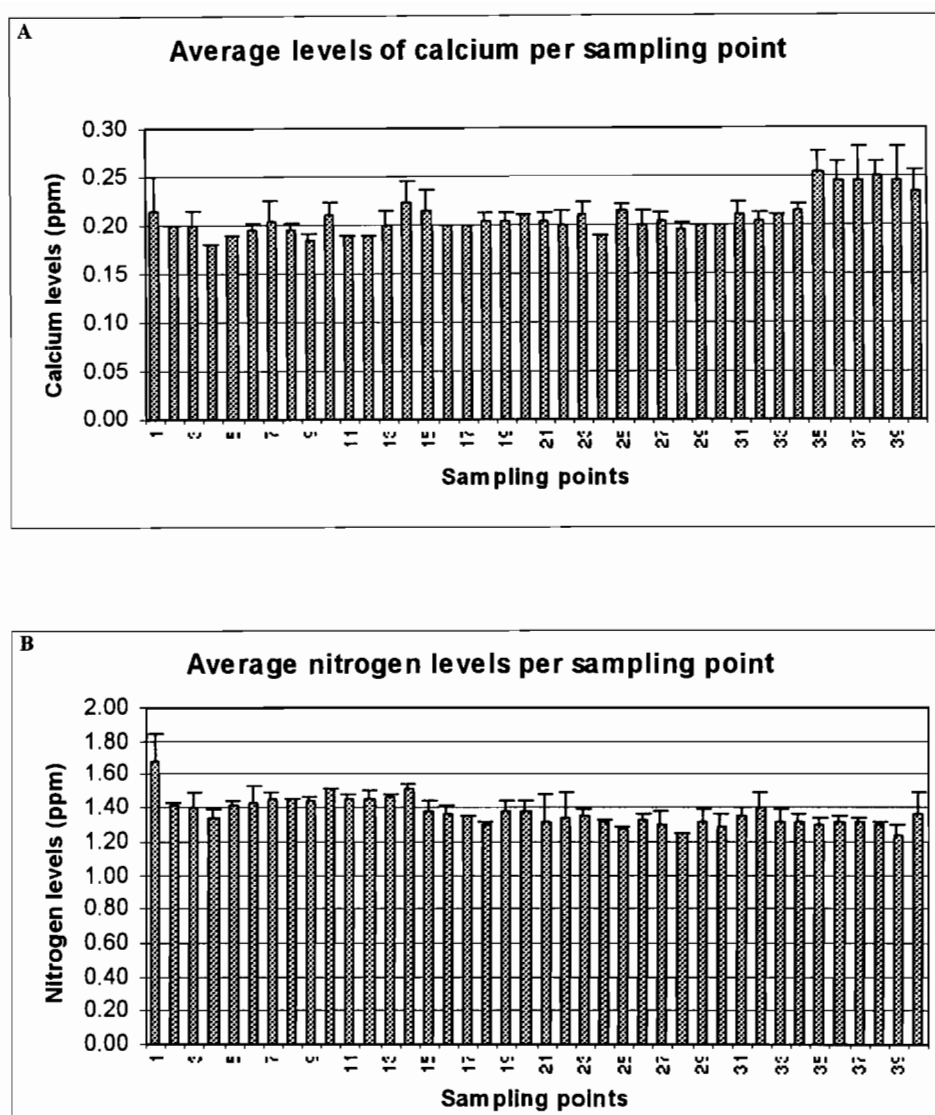


Figure 4: Differences in nitrogen (A) and calcium (B) levels at the 40 sampling points along transects1 & 4.

B. NEMATODE DISTRIBUTION IN RELATION TO LEAF ELEMENTS.

In the previous section, when the spatial distribution of leaf elements was determined, two zones along the transects were seen. The first zone was dominated by leaves with high nitrogen levels while the second zone was dominated by leaves with high calcium levels. In this study, the nematode species and leaf element data obtained from the same sampling points were analysed together to determine whether a relationship existed between some nematode species and leaf elements.

7.4 MATERIALS AND METHODS

See chapter 3, section 3.7.

7.5 RESULTS

Rows 1, 26 and 27 were removed (See chapter 3 sections 3.5 and 3.7). Principal Component Analysis performed on the nematode data showed that the factorial map described 30.2 % and 20.9 % of the variability for F1 and F2 respectively. The correlation circle showed that the variables corresponding to *P. zeae* and *X. elongatum* correlated with positive values of F1 whereas *H. dihystra* and *P. minor* correlated with negative values (Fig. 5a). The variables for *S. brachyurus*, *Criconebella* spp. and *N. brevistilus* correlated with negative values of F2.

The PCA performed on leaf data showed that the factorial map described 34.1 % and 18.7 % of the variability for F1 and F2 respectively. The variables corresponding to Zn, Cu, P, Mg, K and N were correlated with positive values of F1 while Ca and Fe were correlated with negative values of F1 (Fig. 5b). Variables corresponding to Mn and S had a high correlation with the negative part of F2.

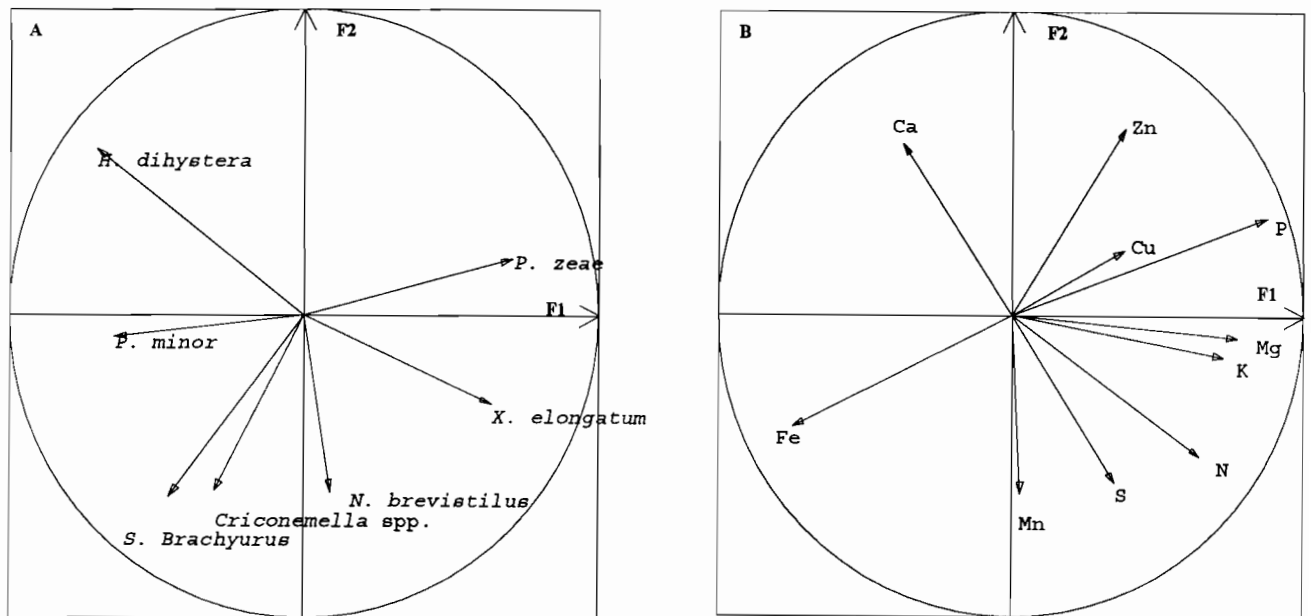


Figure 5: Correlation circles (F1 X F2) of the PCA on nematode species (A) and leaf element data (B) for the 37 sampling points in transects 1 and 4.

A comparison between the distribution of the nematodes on a PCA factor map and on a Three-Ways Analysis compromised factor map show them to be similar, except the species that were on the positive part of F1 on the compromised factor map are now on the negative part and vice versa (Fig.6). Also, *S. brachyurus* now occurs on the negative part of F2 instead of the positive part.

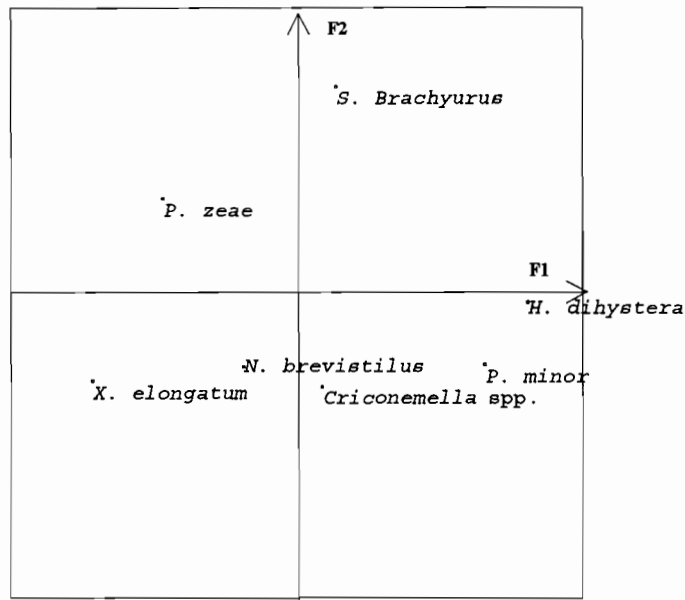


Figure 6: Compromised factorial map (F1 X F2) of the Three-Ways Table Analysis on nematode variables for the 37 sampling points in transects 1 and 4.

Prior to coinertia analysis, a permutation test showing whether the relationship between two tables is significant or not, was computed. The permutation test run between the nematode and leaf datasets showed that the relationship between them was statistically significant, i.e. the nematode species had a correlation with leaf elements, but could be positive or negative, $p < 0.000$ (Fig. 7).

number of random matching: 1000 Observed: 4.901693
 Histogramm: minimum = 0.321223, maximum = 4.901693
 number of simulation X<Obs: 1000 (frequency: 1.000000)
 number of simulation X>=Obs: 0 (frequency: 0.000000)

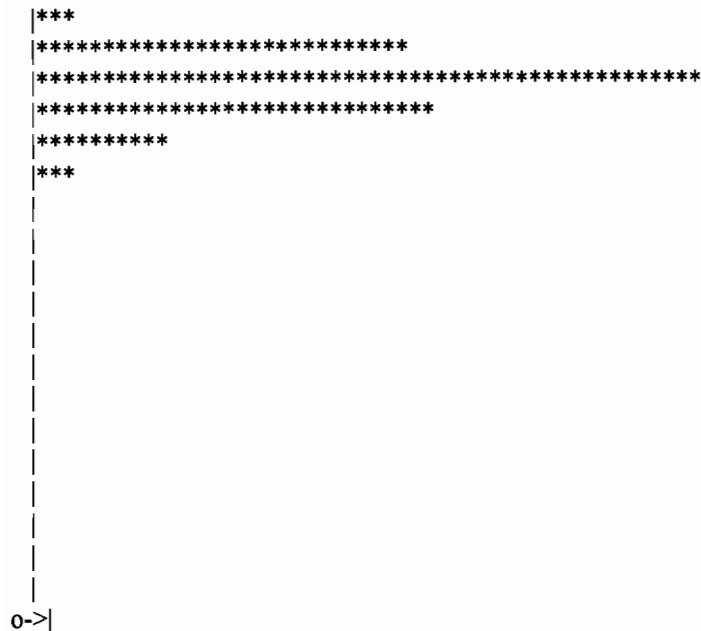


Figure 7: Permutation test of the coinertia analysis for the nematode species and leaf element data for the 37 sampling points in transects 1 and 4.

The permutation test was significant and therefore coinertia analysis was performed. The coinertia factor maps drawn from two datasets highlight a link amongst the variables from the two. They differ from PCA factor maps because they show a correlation between variables from two different datasets whereas PCA factor maps show an association amongst variables from the same dataset.

The nematode coinertia factor map showed that the variables corresponding to *S. brachyurus* had high negative F1 factorial values while the variables corresponding to *P. zae* and *X. elongatum* had positive factorial values along this axis (Fig. 8a). Variables corresponding to *P. minor* and *H. dihystra* had an association with the negative F2 values. The variables corresponding to *N. brevistilus* and *Criconemella* spp. occurred at the centre and their values were very low.

The leaf element coinertia map showed that the variables corresponding to P, Mg and Cu had negative F1 factorial values while variables corresponding to Fe had high positive F1 factorial values. Variables corresponding to Zn and Cu had negative F2 values while variables corresponding to N and K had positive F2 factorial values. Variables corresponding S and Mn had low factorial values and occurred almost at the centre (Fig. 8b).

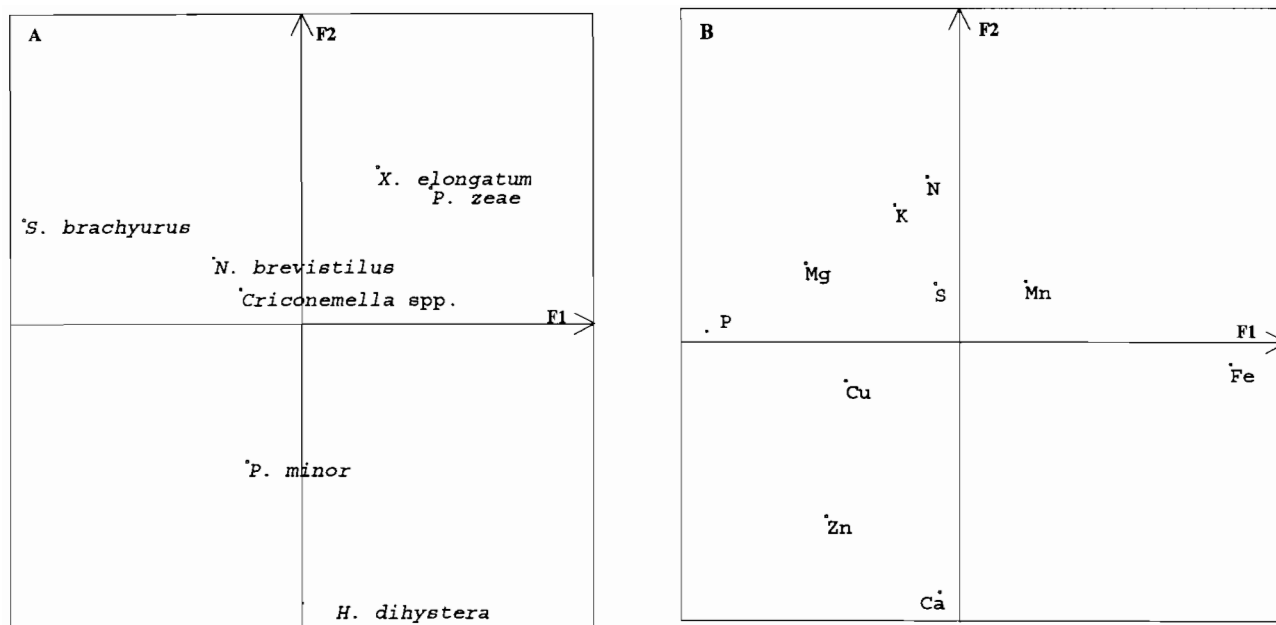


Figure 8: Factor maps (F1 X F2) of the coinertia analysis on nematode species (A) and leaf element data (B) for the 37 sampling points in transects 1 and 4.

A crossed table for the two datasets was drawn up to summarise the correlation between nematodes and leaf elements (Fig. 9). The circles show a positive correlation while the squares show a negative correlation. *Helicotylenchus dihystra* showed a negative correlation with N, P, K, S, Mg and Mn while *X. elongatum* showed a negative correlation P, Ca, Mg, Zn and Cu. *Pratylenchus zeae* had a negative correlation with most elements except N, K, Mn and Fe while *S. brachyurus* and *N. brevistilus* had a positive correlation with almost all elements except Ca, Mn and Fe. *Paratrichodorus minor* had a negative correlation with most of the elements except P, Ca and Zn.

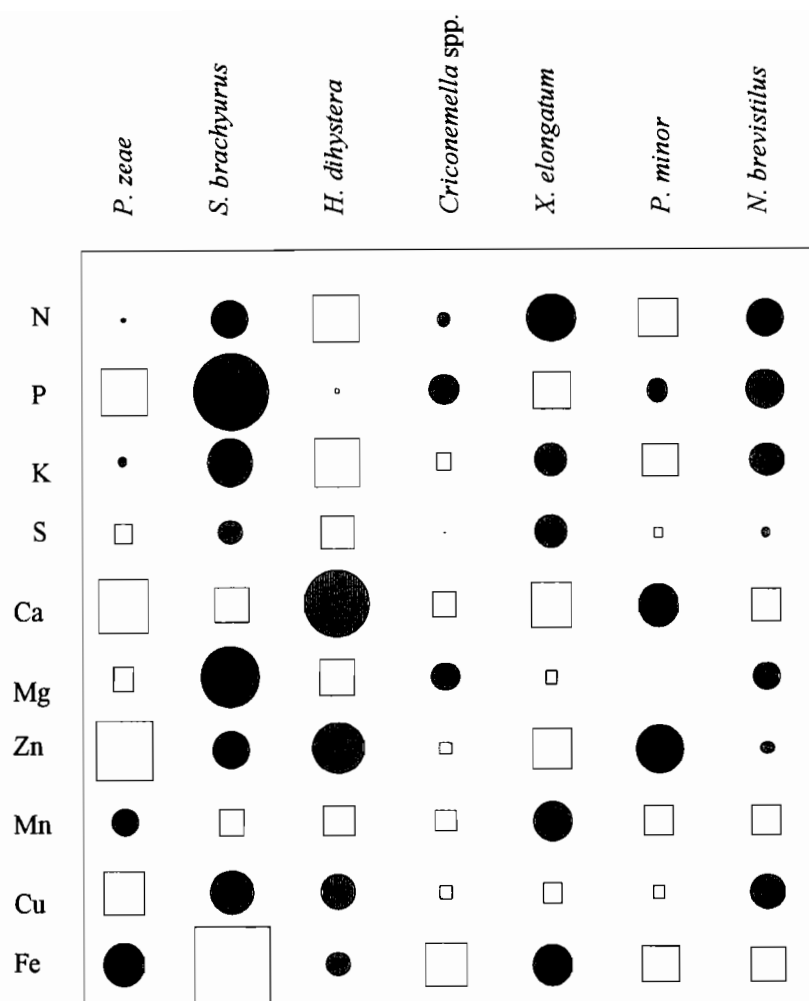


Figure 9: Crossed coinertia table showing the relationship between nematode species and leaf elements in transects 1 and 4. Circles represent a positive correlation while squares represent a negative correlation. The size of the symbol is proportional to the absolute value.

Scatter plots drawn for *H. dihystra* against nitrogen (a) and *X. elongatum* against calcium (b), the regressions were both negative (Fig. 10). The negative regression between *H. dihystra* and nitrogen showed that as nitrogen levels increased, *H. dihystra* % decreased (Fig. 10a). The y-intercept was at 137.32 % while the x-intercept was at 1.56 ppm. The correlation coefficient, $r = 0.61$, showed a strong correlation between the two variables. In Fig. 10b, the regression line cut the y-axis at 96.4 % while the x-intercept was at 0.29. The correlation coefficient, $r = 0.63$ showed a strong correlation between the two variables.

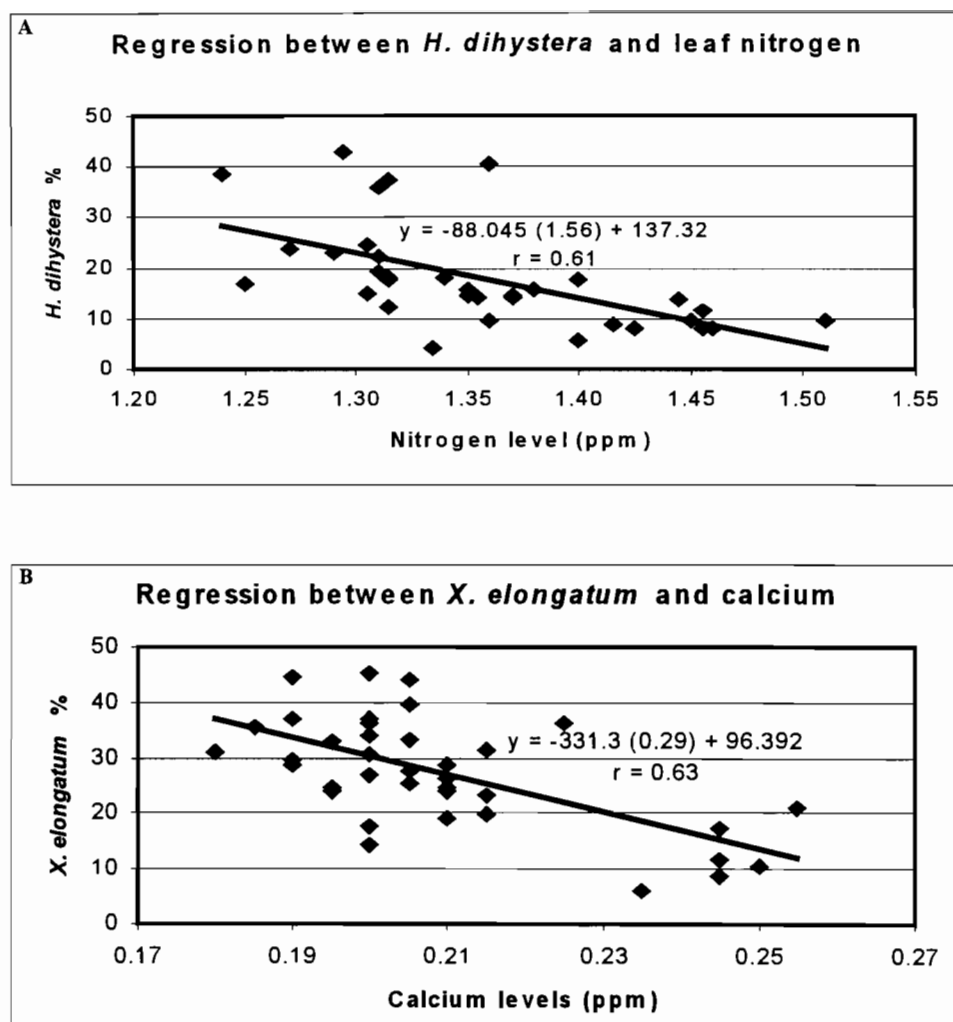


Figure 10: Scatter diagrams showing negative regressions between *H. dihystra* and nitrogen (A) and between *X. elongatum* and calcium (B) for the 37 sampling points.

C. RELATIONSHIP BETWEEN LEAF ELEMENTS AND ABIOTIC SOIL FACTORS

The previous sections of this study showed that there was a correlation between nematode species and soil elements (Chapter 6) and between nematode species and leaf elements. Therefore, soil and leaf elements were analysed together to determine if there was a relationship between them and the way they were distributed along the transects.

7.6 MATERIALS AND METHODS

See chapter 3, section 3.8.

7.7 RESULTS

Rows 1, 26 and 27 were removed (see Chapter 3 sections 3.5 and 3.7). Both soil and leaf data were analysed using PCA before coinertia analysis was performed. Principal Component Analysis performed on the soil data with 37 rows showed that the factorial map described 29.3 % and 14.3 % of the variability for F1 and F2 respectively. The correlation circle showed that the variables of clay and medium sand had high positive factorial values on F1. The variables corresponding to pH, Mg, Ca, K and silt had high negative factorial values and occurred on the negative part of F1 (Fig. 11a). The variables corresponding to coarse sand occurred on the negative side of F2 while those corresponding to Fe and S occurred on the negative side of F2.

Principal Component Analysis performed on leaf data with 37 rows showed that the factorial map described 34.1 and 18.7 % of the variability for F1 and F2 respectively. In the leaf variables' correlation circle, the leaf elements showed size effect. The variables corresponding to the Fe and Ca occurred on the negative part of F1 while P, Mg and K had positive factorial values along F1. Variables corresponding to Mn and S occurred on the negative side of F2 (Fig. 11b).

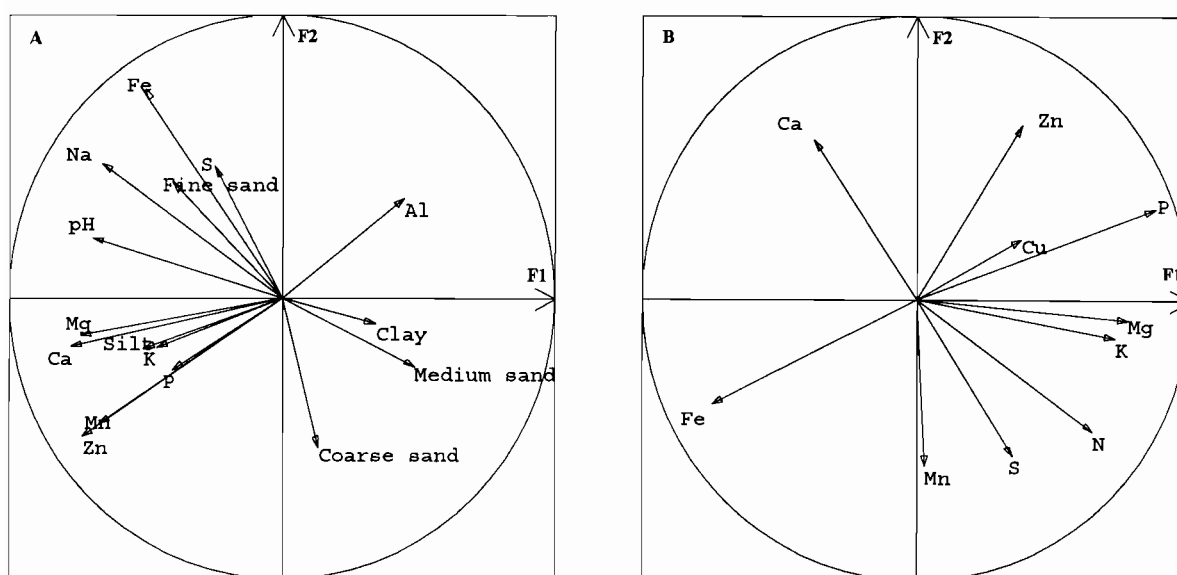


Figure 11: Correlation circles (F1 X F2) of the PCA on soil (A) and leaf element data (B) for the 37 sampling points in transects 1 and 4.

Prior to coinertia analysis, a permutation test between the soil and leaf data was computed. This test shows whether the relationship between two tables is significant or not. The permutation test run between the two datasets showed that the relationship between them was statistically significant, i.e. the soil elements had a

correlation with the leaf elements, $p < 0.000$ (Fig. 12). The rows from the two data sets were randomly matched a 1000 times but the relationship between them remained statistically significant. The observed value therefore applies over the set of random permutation.

number of random matching: 1000 Observed: 10.411654
 Histogramm: minimum = 0.946149, maximum = 10.411654
 number of simulation $X < \text{Obs}$: 1000 (frequency: 1.000000)
 number of simulation $X \geq \text{Obs}$: 0 (frequency: 0.000000)

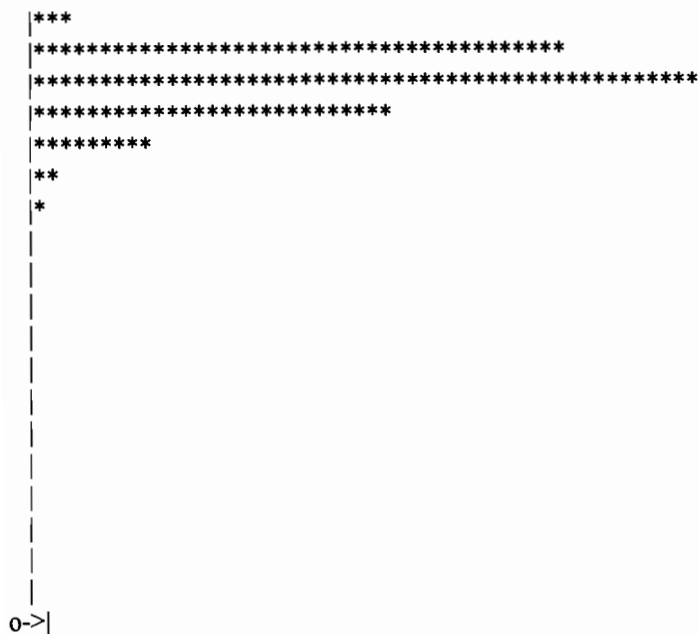


Figure 12: Permutation test of the coinertia analysis for the soil and leaf element data.

Because the permutation test between soil and leaf element data was significant, coinertia analysis was performed. The coinertia factor maps drawn from two independent datasets highlight a link amongst the variables from these data. They differ from PCA factor maps because they show a correlation between variables from two independent datasets, while PCA factor maps show an association amongst variables from the same dataset.

The coinertia factor map resulting from the analysis of the soil data showed that the variables corresponding to Na, pH, S and Fe had high negative F1 factorial values while the variables corresponding to clay had positive factorial values along the first axis (Fig. 13a). The variables corresponding to coarse sand had positive F2 values while variables corresponding to silt had negative F2 values. The variables corresponding to Al occurred at the centre and their values were very low. The coinertia maps were used

because they highlighted the correlation between the soil and element variables instead of PCA factor maps, which showed an only the association amongst the soil elements.

The leaf element coinertia map showed that the variables corresponding to P had high negative F1 factorial values while those corresponding to Fe had high positive F1 factorial values. Variables corresponding to N, K and S had high positive F2 factorial values while variables corresponding to Ca had high negative F2 values. Variables corresponding to Mn and Cu had low factorial values and occurred almost at the centre (Fig. 13b). Even here, PCA factor maps could not be used as they show an association amongst the leaf elements and not a correlation between soil and leaf elements.

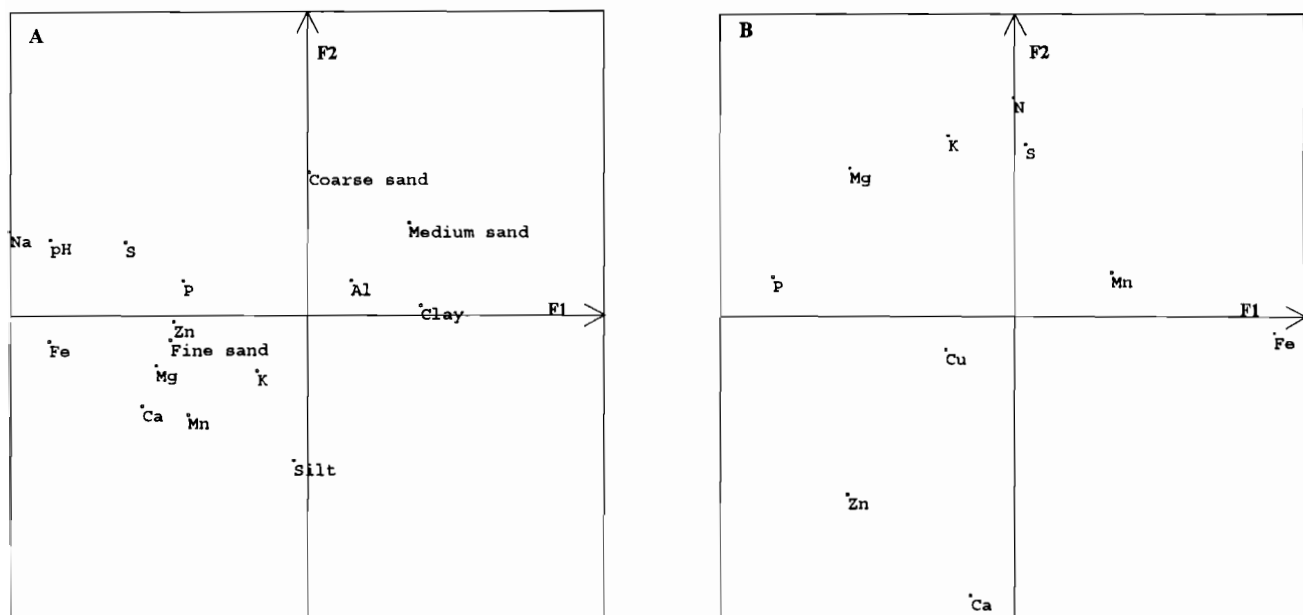


Figure 13: Factor maps (F1 X F2) of the coinertia analysis on soil (A) and leaf element data (B) for the 37 sampling points in transects 1 and 4.

A crossed table for the two datasets was drawn to summarise which soil elements had a positive or negative relationship with which leaf elements (Fig. 14). The circles show a positive correlation while the squares show a negative correlation. In the soil, Na, pH and Fe had strong positive correlations with P in leaves. Clay and medium sand had positive correlations with leaf Fe. Silt had a positive correlation with leaf Ca while coarse sand had a positive correlation with N. Iron in soil had a strong negative correlation with leaf Fe while soil P and Mg had positive correlations with their counterparts in leaves.

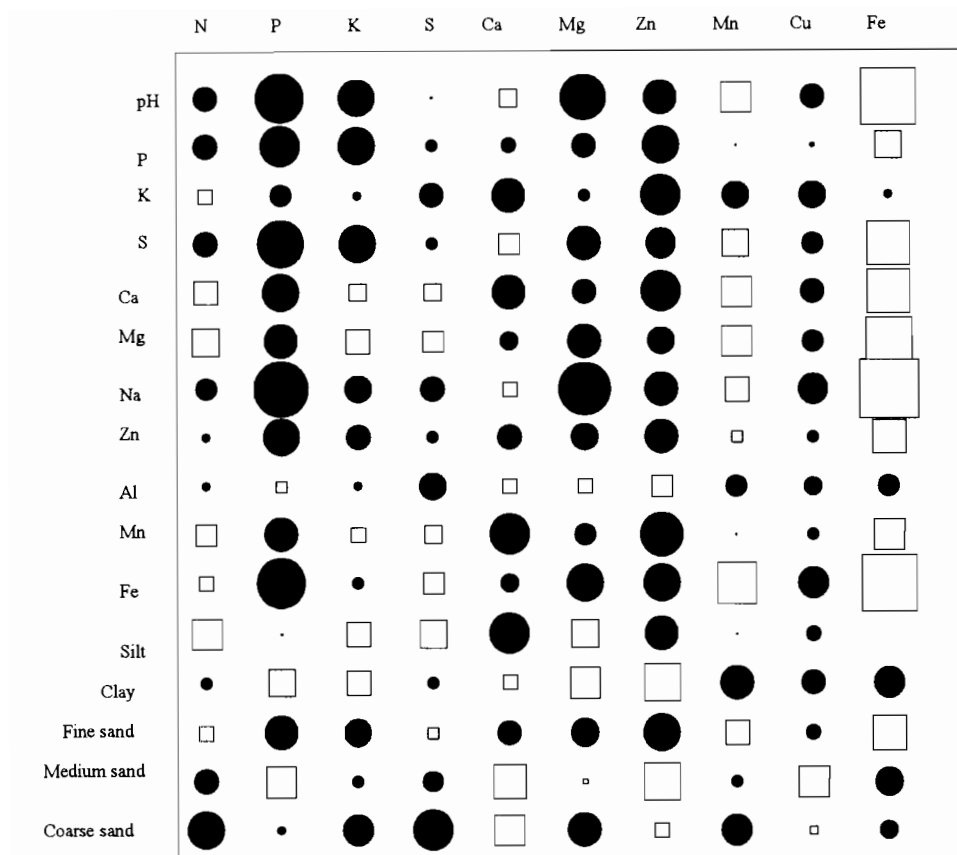


Figure 14: Crossed coinertia table showing the relationship between soil and leaf elements. Circles represent a positive correlation while squares represent a negative correlation. The size of the symbol is proportional to the absolute value.

When scatter plots for clay against leaf Mg (a), coarse sand against leaf Ca (b), silt against leaf N (c) and soil Fe against leaf Fe (d) were drawn, the regressions were negative (Fig. 15). These soil characteristics and leaf elements were chosen because they had high factorial values and showed a strong correlation amongst them. In Fig. 15a, the regression line cut the y-axis at 0.12 ppm and the x-value at 81.27 %. The correlation coefficient, $r = 0.17$ showed a weak correlation between clay and leaf magnesium. The regression coefficient was negative implying that as clay content increased, magnesium levels in leaves decreased. In Fig 15b, the y-intercept was at 0.22 ppm and the x-intercept was at 15.68 %. The negative regression coefficient implied that calcium in leaves increased as coarse sand content decreased. The correlation coefficient, $r = 0.25$ showed a correlation between the two variables. In Fig. 15c, the y-intercept was at 1.5 ppm while the x-intercept was at 43.6 %. The correlation coefficient, $r = 0.41$ showed a strong

correlation between silt content and nitrogen in leaves. The slope, $b = -0.0349$, implied that as silt content increased, nitrogen levels in leaves decreased. In Fig. 15d, the regression line cut the y-axis at 111.41 ppm while the calculated x-intercept was at 1578 ppm. The negative regression coefficient, $b = -0.0706$ implied that as levels of soil iron increased, the levels of leaf iron decreased. The correlation coefficient, $r = 0.42$, showed a strong correlation between soil and leaf iron.

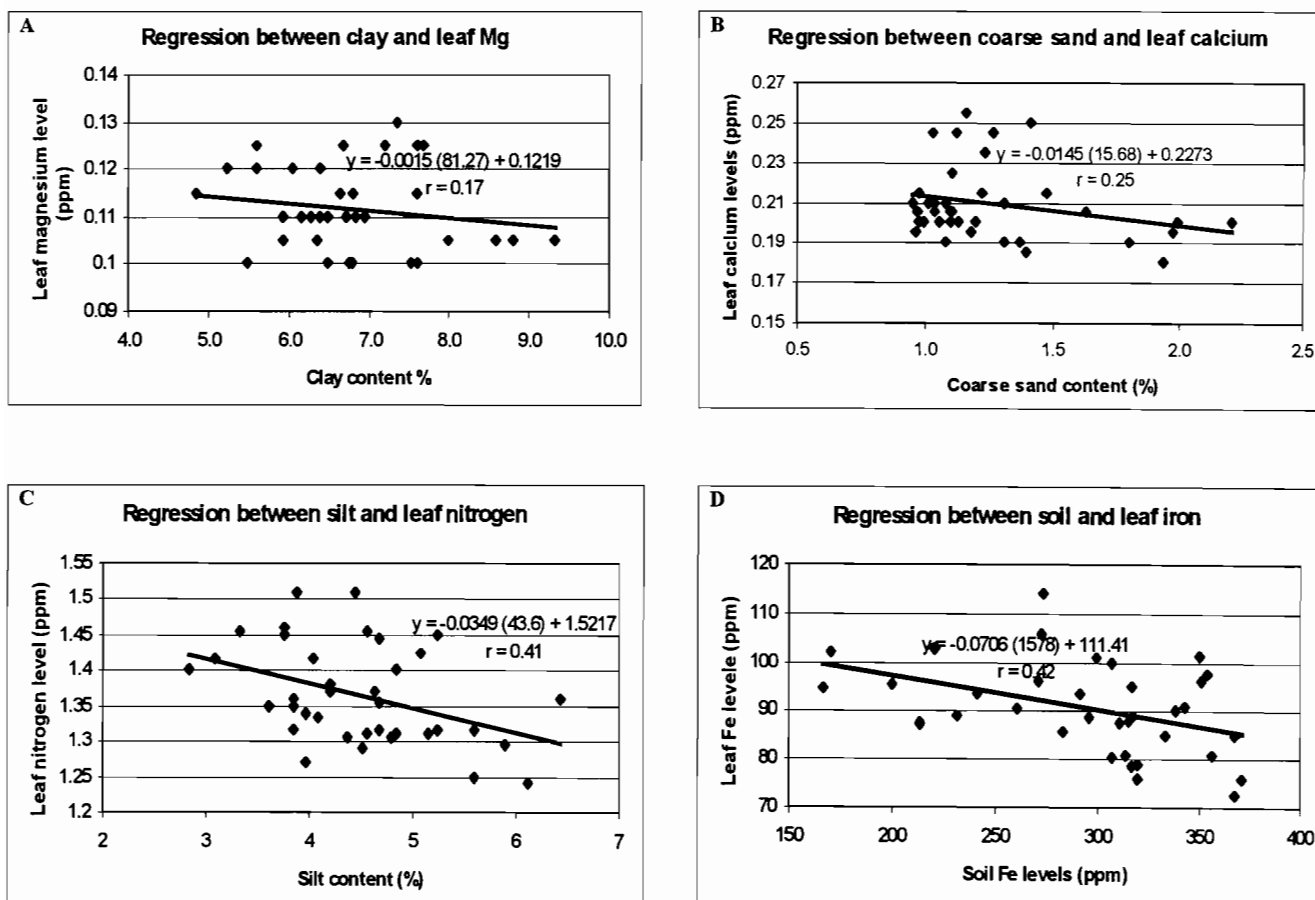


Figure 15: Scatter plots showing negative regressions between clay and leaf Mg (A), coarse sand and leaf Ca (B), silt and leaf N (C) and between soil Fe and leaf Fe (D).

7.8 DISCUSSION

Efficiency in nutrient uptake differs amongst plants in a field, depending on availability and efficiency of their root systems. This was shown in this study by the high values of N at the first sampling point of the fourth transect. The two homogenous zones on the two transects indicates that the spatial distribution of leaf elements remained stable. The occurrence of all the leaf elements on the positive side of the first factor, except Fe and Ca that occurred on the negative part of the second factor, showed size effect although Ca and Fe were positively correlated with the other elements.

There was a difference in the distribution of leaf elements along each transect, with the first half dominated by leaves with high N content and the second half by leaves with high Ca content. As the N and Ca levels in the leaves differed over the first and second halves of the transects respectively, a relationship between leaf elements and nematodes was identified. Nematodes also showed differences in their distribution per sampling point along the transects.

In the study between nematodes and leaf elements, high proportions of *X. elongatum* were found at sampling points with high nitrogen levels in leaves while the opposite was true for *H. dihystrera*. Plants with high levels of Ca in their leaves grew in areas with high proportions of *H. dihystrera* and low percentages of *X. elongatum* while plants with high levels of leaf N grew in areas with high *X. elongatum* percentages. At sampling points where leaves had high levels of P, Mg and Cu, high proportions of *S. brachyurus* were also found.

Theoretically, the scatter plots between nematodes and leaf elements showed that sampling points with zero ppm nitrogen levels had maximum *H. dihystrera* percentages while sampling points with 1.56 ppm nitrogen levels, no *H. dihystrera* was found (Fig. 10a). In Fig. 10b, the occurrence of the y-intercept at 96.4 % and the x-intercept at 0.29 ppm implies that at sampling points with 0 ppm calcium levels had 96.4 % of *X. elongatum* and at sampling points with 0.29 ppm levels of calcium, there was no *X. elongatum* found.

Soil physical properties and leaf elements showed a strong correlation amongst them. Leaf Ca had a strong positive correlation with silt and a negative one with coarse sand, implying that plants grown in soils with high silt content had high levels of calcium in their leaves while those grown in high coarse sandy soil had low leaf Ca levels. Plants with high nitrogen levels in their leaves grew in areas with high levels of coarse sand and the reverse was true for those that grew in silt soils.

In Fig. 15a, the y-intercept at 0.12 ppm while the x-intercept at 81.27 % imply that in sampling points where the clay content was 0%, the magnesium level in leaves was 0.12 ppm. Theoretically, leaves collected from sampling points with 81.27 % clay would have no magnesium in them. The negative

regression line with the y-intercept at 0.23 ppm and x-intercept at 15.68 % in Fig. 15b implies that in sampling points with 0% coarse sand, the calcium level in leaves was 0.23 ppm while in points with 15.68 % coarse sand, the leaves had no calcium in them. In Fig. 15c, the y-intercept was at 1.52 ppm while the x-intercept was at 43.6 %. This implies that sampling points with 0% silt had 1.52 ppm nitrogen in their leaves while leaves in sampling points with 43.6 % silt had no nitrogen in them. The regression line for soil and leaf iron in Fig. 15d cut the y-axis at 111.41 ppm while the x-intercept was at 1578 ppm. Theoretically, this implies that when soil iron is at 0 ppm, leaves have 111.41 ppm while when soil has 1578 ppm of iron, leaves have no iron in them.

High percentages of *X. elongatum* were found in areas with high P, medium and coarse sand levels while high *H. dihystra* proportions occurred in areas with high Mg, Ca, silt, clay and fine sand levels. In the study of leaf element distribution, the leaves from the first part of the transects had high N levels while the ones from the second part had high Ca levels. The study between nematode species and leaf elements showed that leaves with high levels of Ca, Zn and Cu were found in areas with high percentages of *H. dihystra* while leaves with high levels of N were found in areas with high proportions of *X. elongatum*. The study between soil and leaf elements showed that high leaf Ca, Zn and Cu levels were found in soil with high silt and clay contents, the same soil that had high *H. dihystra* proportions. Plants with high leaf P levels grew in soil with high coarse sand content, the same soil where high proportions of *X. elongatum* were found.

CHAPTER 8

MANIPULATION OF NEMATODE COMMUNITIES FOR LESS PATHOGENIC SPECIES THROUGH THE USE OF ORGANIC AMENDMENTS

8.1 INTRODUCTION

Most of the agricultural land in the world is still cultivated using traditional methods (Bridge, 1996). In sub-Saharan Africa, according to Stifel (1989), small-scale and subsistence farmers using traditional methods are generally more productive per unit of land than are large-scale farmers as their methods are designed to conserve as much of the essential soil goodness as possible. This is also true for sugarcane as it is easier for a farmer to manage and take care of a small field than a large piece of land. Many practices used in traditional agriculture help in reducing nematodes and other pests, either totally or in a modified form (Bridge, 1996), e.g. use of nematode-free planting material, hot-water treatment of planting material, crop rotation, fallowing the land, destruction of infected crop residue and use of organic amendments. Small-scale and subsistence farmers use traditional methods to avoid the buildup of parasitic nematode population densities. In Nigeria, *Meloidogyne incognita* is controlled by the addition of cow dung and poultry manure (Poswal & Akpa, 1991). In India, neem cake and press mud were used to control *Hirschmaniella* spp. in rice fields (Johnathan & Pandiarajan, 1991).

In many rural communities of South Africa, composting is not a common practice although there is a huge demand for food production and soil conservation, and because financial constraints often make the use of chemical fertilisers and pesticides almost impossible (Smith & Hughes, 2002). Compost and sludge amendments improve physical and chemical soil properties (Smith & Hughes, 2002, Diehn & Zuercher, 1990), enhance soil water retention (McSorley & Gallaher, 1995a, Villenave & Cadet, 1999), reduce soil compactibility (Bazzoffi, et al., 1998) and enhance biological activity (Pfoetzer & Schöler, 1997). Composts can protect plants from soil borne pathogens (Schöler et al., 1993).

Pulp and paper mill sludge, when used as a soil amendment, contributes to the organic matter content which may result in chemical and physical changes in soil (Vagstad et al., 2001). Waste sludges from paper mills have been used as soil amendments for a variety of crops (Hughes & Girdlestone, 2001). Sugarcane trash contains large quantities of dry matter and nutrients (Thorburn et al., 2001). Eighty to 95 % of dry matter and nitrogen are lost, with lower losses of other nutrients, when sugarcane is burnt (Mitchell et al., 2000). Harvesting cane while it is still green and retaining the trash blanket has a considerable effect on nutrient cycling (Thorburn et al., 1999, Thorburn et al., 2001).

Organic amendments not only reduce plant parasitic nematode populations but are also rich in nutrients, improve soil physical and chemical properties and also its water-holding capacity. Unlike nematicides, they are inexpensive and depending on the amount of effluent in them, they are less harmful to the environment. This study was conducted to determine whether the use of organic amendments protected sugarcane roots from plant parasitic nematode attacks as well as influenced the nematode communities by manipulating them for the mildly pathogenic species, *Helicotylenchus dihystera*. The hypothesis is that high numbers of endoparasites decrease root weights and hence shoot germination. Sett roots are most vulnerable during the germination period and attacks by nematodes affect germination as some buds may abort or take longer to germinate. This may lead to few shoots developing and those that develop later may face competition for food and light from the older shoots. According to Villenave & Cadet (1998), *H. dihystera* had a neutralising effect on other nematode community species in millet and results from West Africa seem to agree with this phenomenon on sugarcane (Cadet et. al, 2001).

8.2 MATERIALS AND METHODS

See chapter 3, section 3.9.

8.3 RESULTS

8.3.1 EFFECT OF TREATMENTS ON ROOT INFESTATION

8.3.1.1. SETT ROOTS

The infestation of sett roots under the different treatments was significantly different, $p \leq 0.05$, with aldicarb and thume + filter cake-treated plots having the lowest endoparasite numbers but showing no significant difference between themselves. Plots treated with filter cake, extra filter cake + furfural and trash + filter cake had significantly higher endoparasite numbers than aldicarb and thume + filter cake-treated plots although they showed no difference amongst themselves. Sett roots in control and filter cake + furfural-treated plots showed no significant difference between themselves and had the highest numbers of endoparasites (Fig. 1). The endoparasite numbers in filter cake, trash + filter cake and extra filter cake + furfural-treated plots showed no significant differences while also those in aldicarb and thume + filter cake-treated plots were not significantly different either.

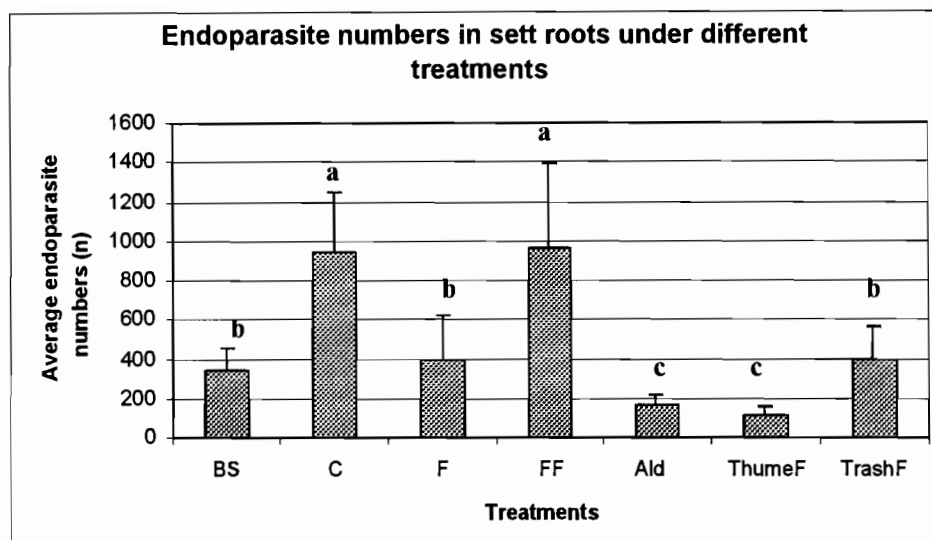


Figure 1: Sett root infestation by endoparasitic nematodes under seven different treatments. BS = extra filter cake + furfural, C = control, F = filter cake, FF = filter cake + furfural, Ald = aldicarb, thumeF = Thume + filter cake, TrashF = trash + filter cake. Bars with the same letter on top show that the difference of endoparasite numbers in those treatments was non-significant.

8.3.1.2. SHOOT ROOTS

A. GERMINATION PERIOD

The infestation of shoot roots by endoparasites under the seven treatments was significantly different, $p \leq 0.05$. In thume + filter cake, trash + filter cake and extra filter cake + furfural-treated plots, endoparasite numbers were lower than and significantly different from endoparasites numbers in aldicarb, filter cake and filter cake + furfural-treated plots (Fig. 2). The three latter treatments had a non-significant difference in numbers of endoparasites found in their roots. Control plots had the highest and significantly different endoparasite numbers in their shoot roots compared to the other treatments.

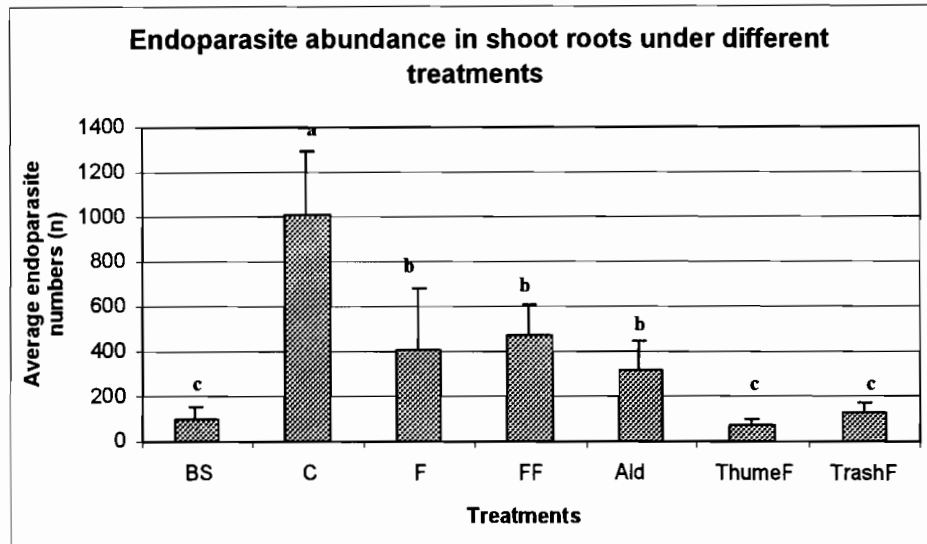


Figure 2: Differences in average numbers of endoparasites/gram of shoot roots under the seven treatments. Bars with the same letter are not significantly different. BS = extra filter cake + furfural, C = control, F = filter cake, FF = filter cake + furfural, Ald = aldicarb, ThumeF = thume + filter cake, TrashF = trash + filter cake.

B. WHOLE CROP CYCLE

When the data on endoparasite numbers in shoot roots from the different sampling dates were accumulated according to the different treatments, control and plots treated with filter cake + furfural had the highest number of endoparasites (Fig. 3). Endoparasite numbers found in trash + filter cake, filter cake, thume + filter cake and extra filter cake-treated plots respectively were less than the numbers found in the control and filter cake + furfural-treated plots. Plots treated with aldicarb had the least number of endoparasites in their shoot roots.

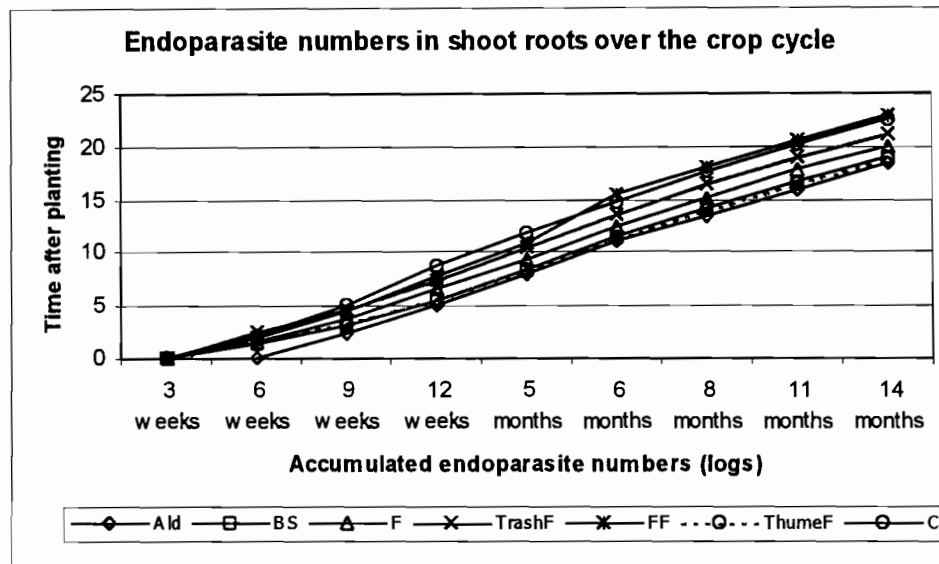


Figure 3: Accumulated endoparasite numbers in shoot roots according to the different treatments during the entire crop cycle. Ald = aldicarb, BS = extra filter cake + furfural, F = filter cake, TrashF = trash + filter cake, FF = filter cake + furfural, ThumeF = thume + filter cake, C = control.

8.3.2 EFFECT OF NEMATODES ON SETT ROOT DEVELOPMENT

When a regression graph between the numbers of endoparasitic nematodes found in sett roots and the average sett root weights was plotted, the regression line was found to be negative, implying that the association between endoparasites and sett root weights was negative (Fig. 4). As endoparasite numbers increased in the sett roots, the weights of the roots decreased. The regression line had a correlation coefficient equal to 0.13, with $n = 49$, showing that the association between endoparasites and sett root weights was not very close. The equation of the regression line showed that the y-intercept, which is the value where the regression line cuts the y-axis and gives the value of y for x , was at 0.26 g. When the x-intercept was calculated, the regression line was found to cut the x-axis at 8.22. The regression coefficient (slope = 0.03) was negative, showing that as endoparasite numbers in sett roots increased, the sett root weights decreased but was non-significant.

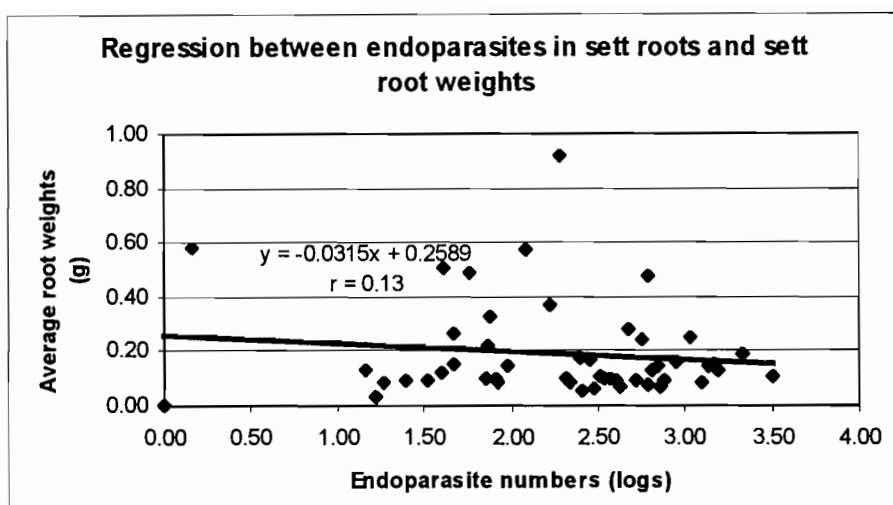


Figure 4: Regression between endoparasitic nematodes and average sett root weights for the 49 plots represented by dots. Regression equation ($y = bx + a$) shows the y-intercept, a , and the slope, b . The correlation coefficient, r , shows the association between the two variables.

8.3.3 EFFECT OF NEMATODES ON NODE GERMINATION PERCENTAGE

When the regression between average number of endoparasites found in sett roots and germination percentage (number of nodes with germinated shoots over total number of nodes in a sett) of shoots was determined, a negative regression line was found (Fig. 5). The calculated equation of the regression line showed that the y-intercept was at 60.2 %. When the x-intercept was calculated, the regression line was found to cut the x-axis at 6140. The regression coefficient or slope of the line, equal to 0.0098, was negative. The slope shows an increase in the y-axis variable corresponding to a unit increase or decrease in x-axis variable. This implies that as endoparasite numbers increased, shoot germination percentage decreased and vice versa. Germination percentage was the dependent variable as the number of endoparasites found in sett roots determined shoot germination rate. The correlation coefficient, $r = 0.32$ with $n = 49$, showed that the relation between endoparasites in sett roots and germination percentage was significant.

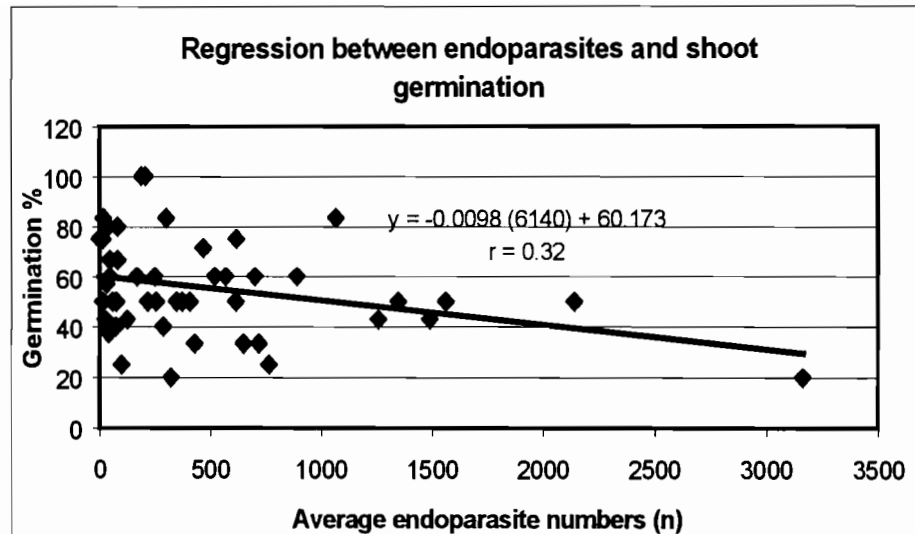


Figure 5: Regression between endoparasitic nematodes and node germination percentage for the 49 plots, represented by the points along the regression line. Regression equation ($y = bx + a$) shows the y-intercept, a, and the slope, b. The correlation coefficient, r, shows the association between the two variables.

When the regression between sett root weights and germination percentage was determined, a positive regression line was found (Fig. 6). The calculated equation of the regression line showed that the y-intercept was at 50.3%. When the x-value was calculated, the y-value was found to be 100 when the x-value was 1.79. As regression shows an increase or decrease of one variable in relation to another, germination percentage was the dependent variable while sett root weights were the independent variable. The regression coefficient was equal to 27.62 and was positive. This implies that an increase in sett root weights led to an increase in shoot germination percentage. The correlation coefficient, r, between sett root weights and germination percentage was positive.

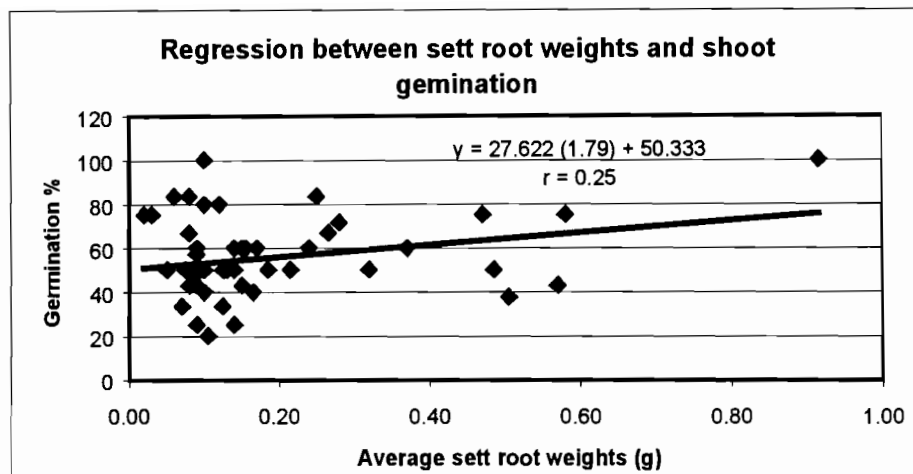


Figure 6: Regression between sett root weights and shoot germination percentage for the 49 plots. Regression equation ($y = bx + a$) shows the y-intercept, a , and the slope, b . The correlation coefficient, r , shows the association between the two variables.

8.3.4 EFFECT OF TREATMENTS ON NEMATODES IN SOIL

Averages of nematode percentage data collected four months after planting until harvesting were calculated and these data was analysed using PCA. Rows 4 and 13 were removed because they had very high *Tylenchorhynchus* spp and *N. brevistilus* numbers respectively which caused these plots to differ from the others when plotted on a factor map. When the soil nematode data with 47 rows corresponding to the 47 plots was analysed using PCA, the factorial map described 29.03 and 17.42 % of the variability for the first (F1) and second (F2) axis respectively (Fig. 7). The variables corresponding to *S. brachyurus* and *H. dihystra* correlated with the negative value of the first factor while variables corresponding to *Tylenchorhynchus* spp., *Meloidogyne* spp., *P. minor*, *P. zae*, *Hoplolaimus* spp. and *Criconemella* spp. were correlated with the positive value of the F1 axis. The variables corresponding to *X. elongatum* and *N. brevistilus* were correlated more with the positive values of the F2 axis, an axis less important than the F1 axis. Variables corresponding to *X. elongatum* were on opposites with variables corresponding to *S. brachyurus* + *H. dihystra*.

8.3 4.1 CORRELATION CIRCLE (Relationship between species)

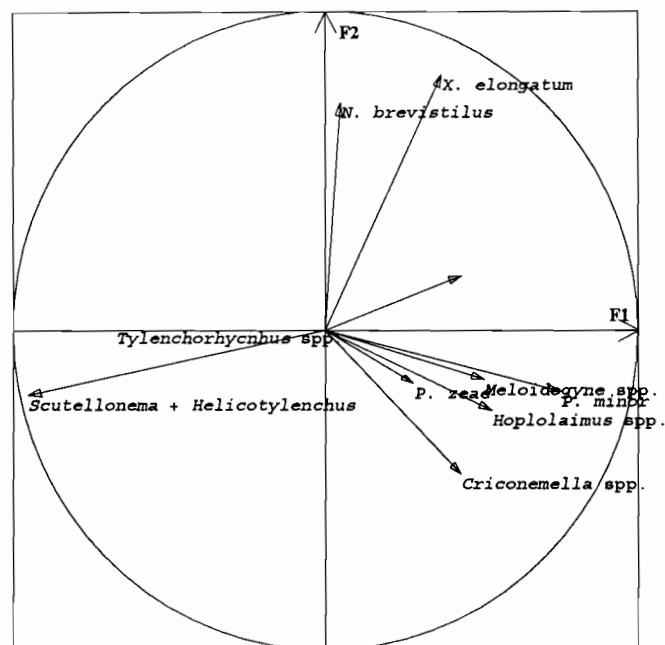


Figure 7: Nematode correlation circle showing relationship between different nematode species. Arrows pointing towards the same direction are positively correlated.

8.3 4.2 FACTORIAL PLAN (Effect of the treatments)

A factorial plan of the seven treatments projected onto nematode percentages was drawn (Fig. 8). This graphic shows each plot linked to the centre of gravity of the other plots with the same treatment. The centres of gravity of plots treated with aldicarb, extra filter cake + furfural, filter cake, trash + filter cake and thume + filter cake occurred on the negative part of the first axis while control and filter cake + furfural-treated plots had their centres of gravity on the positive part of the F1 axis. The seven treatments showed variability amongst themselves as the points representing the seven replicates were scattered along the four quadrants. The nematode data analysed according to the different treatments showed that plots treated with aldicarb (1), extra filter cake + furfural (2), filter cake (3), trash + filter cake (4), and thume + filter cake (6) had high percentages of *S. brachyurus* and *H. dihystra*. Control (7) plots had high percentages of *X. elongatum* and *N. brevistilus*. Filter cake + furfural (5) plots had high percentages of *Meloidogyne* spp., *P. minor*, *P. zeae* and *Tylenchorhynchus* spp.

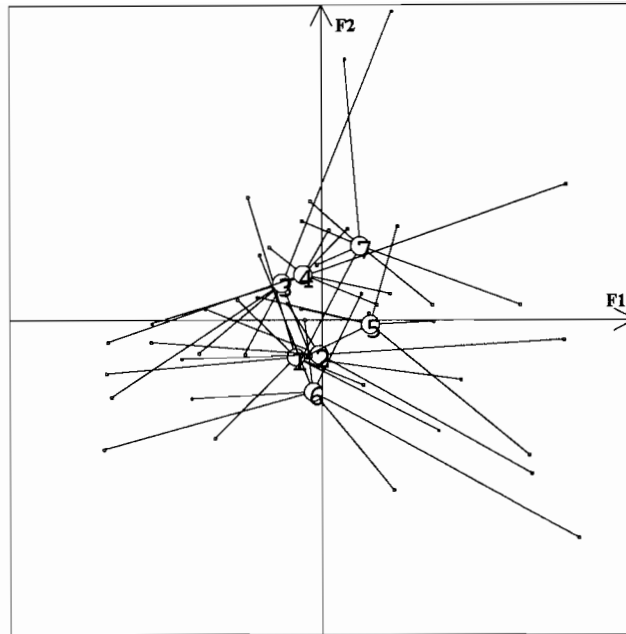


Figure 8: Factor map showing nematode percentages in the 47 plots in relation to the seven treatments. 1 = aldicarb, 2 = extra filter cake + furfural, 3 = filter cake, 4 = trash + filter cake, 5 = filter cake + furfural, 6 = thume + filter cake, 7 = control.

8.3 4.3 DESCRIPTION OF PCA RESULTS WITH ACTUAL NUMBERS

When percentages of *S. brachyurus* + *H. dihystra* were plotted according to the seven treatments, control and filter cake + furfural-treated plots had lower percentages of these species than the other five treatments which did not show a significant difference amongst themselves (Fig. 9a). The numbers in which these species occurred in the different treatments were plotted and control and filter cake + furfural-treated plots had significantly low numbers of these species and showed no significant difference between themselves (Fig. 9b). Thume + filter cake and trash + filter cake had significantly higher percentages of the two species than either control or filter cake + furfural-treated plots and they were not significantly different between themselves. Aldicarb-treated plots had significantly higher percentages of these species than the four former treatments while filter cake and extra filter cake + furfural-treated plots had the highest percentages of these species and there was no significant difference between them.

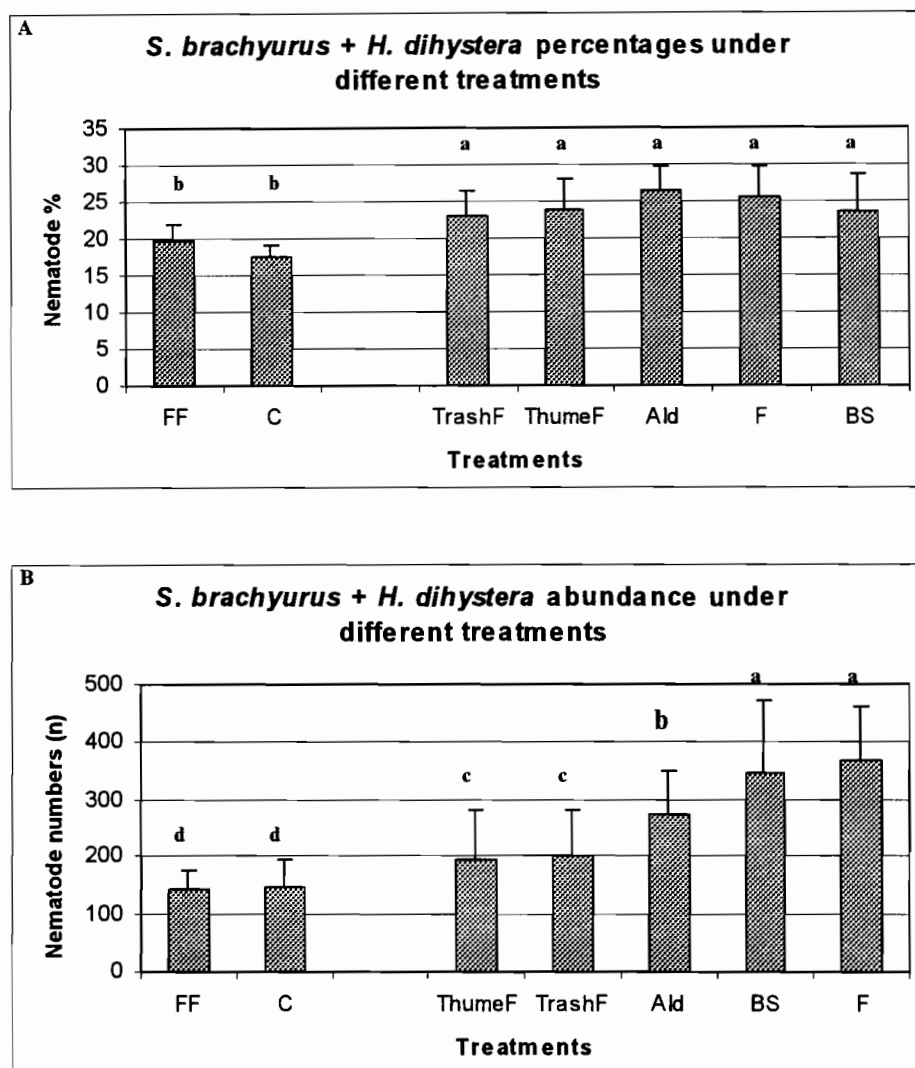


Figure 9: Mean percentage counts of *S. brachyurus* + *H. dihystra* (A) and abundance (B) in the 47 plots corresponding to the seven treatments. Bars with the same letter on top are not significantly different. BS = Extra filter cake + furfural, C = control, F = filter cake, FF = filter cake + furfural, Ald = aldicarb, ThumeF = thume + filter cake, TrashF = trash + filter cake.

8.3.5 RELATIONSHIP BETWEEN NEMATODE COMMUNITIES AND YIELD

Analysis of sugarcane yield, fibre, brix, pol (same as sucrose) and purity data using ANOVA showed that there was no significant difference between the treatments, $p \geq 0.05$. When the actual values of yield (tons/ha) obtained from the different treatments are compared, thume + filter cake treated plots gave the

best yield followed by aldicarb, extra filter cake + furfural, filter cake and filter cake + furfural-treated plots. Trash + filter cake-treated and control plots had the lowest yields. The average and standard error of the means (in brackets) are shown in Table 1.

TREATMENTS	YIELD (TONS/HA)	FIBRE	BRIX	POL	PURITY
Control	79.1 (7.9)	11.6 (0.23)	15.1 (0.33)	13.5 (0.38)	89.5 (0.81)
Aldicarb	86.1 (8.03)	12.4 (0.42)	15.1 (0.13)	13.6 (0.16)	89.9 (0.69)
Filter cake	83.9 (8.8)	11.8 (0.27)	15.1 (0.07)	13.6 (0.13)	90 (0.62)
Filter cake + furfural	82.7 (5.9)	11.8 (0.19)	15.0 (0.16)	13.3 (0.21)	88.7 (0.78)
Thume + filter cake	86.9 (6.1)	11.6 (0.21)	14.8 (0.25)	13.3 (0.28)	89.9 (0.54)
Trash + filter cake	63.8 (6.8)	12.3 (0.29)	15.1 (0.22)	13.5 (0.22)	89.1 (0.86)
Extra filter cake + furfural	85.9 (6.9)	11.8 (0.30)	15.5 (0.20)	13.9 (0.18)	89.7 (0.72)
ANOVA	NS	NS	NS	NS	NS

Table 1: Mean averages of sugarcane yield, fibre, brix, pol and purity in the different treatments. Means of the different treatments are not significantly different.

Although there is no definite treatment that has managed to manipulate nematode communities, high percentages and abundance of *S. brachyurus* and *H. dihystra* were found in all plots except those treated with filter cake and control plots. Yield data was therefore projected onto nematode data to determine if certain nematode species have an effect on yield.

8.3.5.1 YIELD PROJECTED ON A FACTORIAL MAP

The yield data were projected onto nematode rows to determine which plots, dominated by which nematode species, gave the lowest and highest yields (Fig. 10). Plots with variables corresponding to high percentages of *S. brachyurus* + *H. dihystra* had above average yields represented by circles while variables corresponding to *Tylenchorhynchus* spp., *Meloidogyne* spp. *P. zae*, *P. minor*, *Hoplolaimus* spp. and *Criconea* spp. were found in plots with below average yields shown by squares. Most of the circles occurred on the negative part of the first axis and had negative factorial values while the majority of squares occurred on the positive part of F1 axis and had positive factorial values. Variables corresponding to *X. elongatum* and *N. brevistilus* were found in plots with above average yields along the F2 axis. The harmful endoparasites, *P. zae* and *Meloidogyne* spp. were found on the part of F1 with the majority of squares.

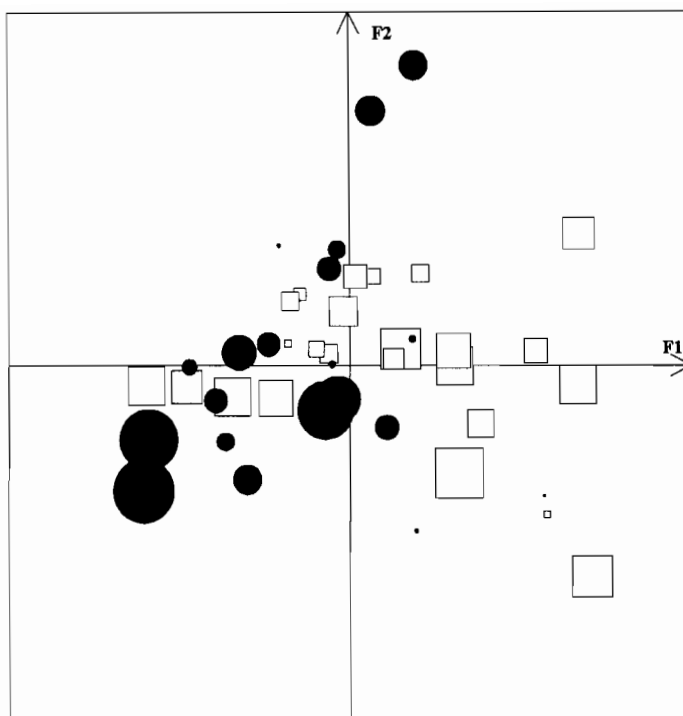


Figure 10: Factor map of sugarcane yield (tons/ha) projected onto 47 nematode plots. Circles represent above average yields while squares represent below average yield.

8.3.5.2 PERCENTAGES OF *S. brachyurus* + *H. dihystra* AND YIELD IN RELATION TO NEMATODE VALUES ON FACTOR MAP

The values obtained from PCA showing the distribution of nematodes on a factorial map were used as a reference to determine plots with highest *S. brachyurus* + *H. dihystra* percentages and yield and plots with lowest *S. brachyurus* + *H. dihystra* percentages and yield (Fig. 11). The 10 highest and 10 lowest values of *S. brachyurus* + *H. dihystra* and the corresponding yield values were used to determine the effect of *S. brachyurus* + *H. dihystra* percentages, both high and low, on yield. High percentages of *S. brachyurus* + *H. dihystra* together with high yield values (tons/ha) corresponded to the negative values of the F1 axis while low *S. brachyurus* + *H. dihystra* percentages and yield values corresponded to the positive values of the F1 axis.

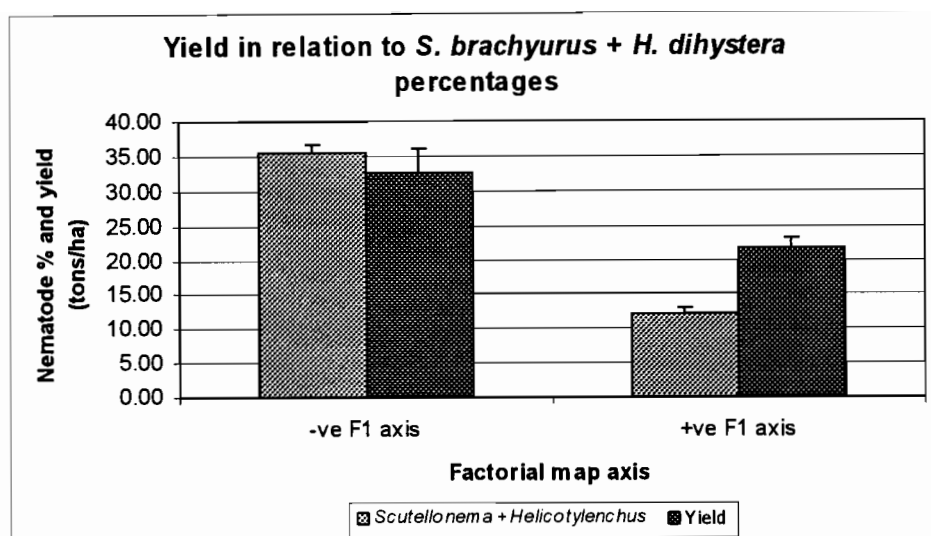


Figure 11: Differences in *S. brachyurus* + *H. dihystra* percentages and yield data (tons/ha) found on the positive and negative parts of the F1 axis.

8.3.5.3 REGRESSION BETWEEN NEMATODE AND YIELD DATA

When the regression between *S. brachyurus* + *H. dihystra* and yield was determined, the regression line found was positive (Fig. 12a). The regression coefficient was positive implying that the yield increased as percentages of *S. brachyurus* + *H. dihystra* increased. The regression line cut the y-axis at 18.84 tons/ha. The correlation coefficient, $r = 0.45$ showed that the association between the two variables was not significant.

When the regression between *Meloidogyne* spp. and yield was determined, the regression line found was negative (Fig. 12b). The regression coefficient was negative implying that the yield increased as percentages of *Meloidogyne* spp. decreased. The y-intercept, where the regression line cut the y-axis, was at 30.06 tons/ha while the calculated x-value, the theoretical number of *Meloidogyne* spp. individuals to cause zero yield, was at 90.71. The correlation coefficient, $r = 0.36$ showed that the association between the two variables was significant.

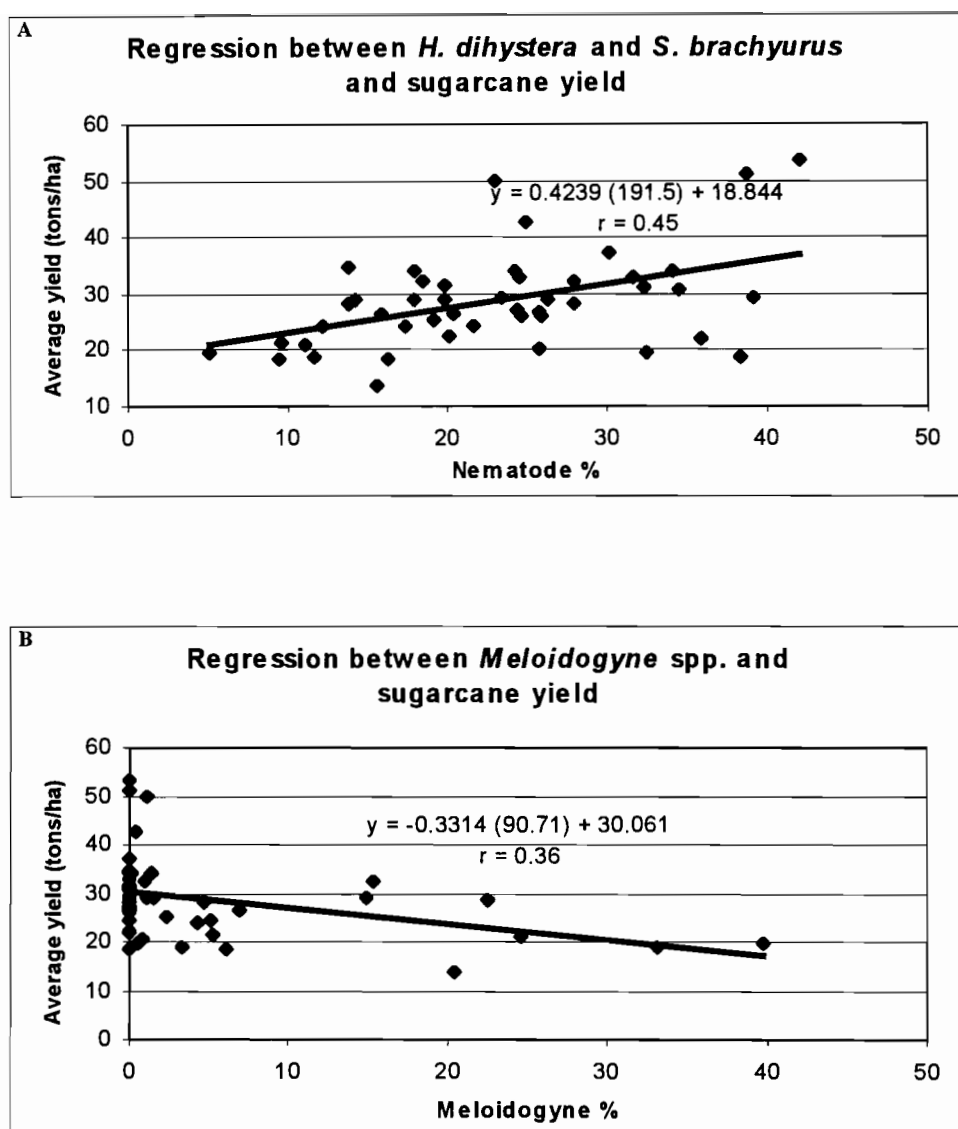


Figure 12: Positive regression between *H. dihystra* + *S. brachyurus* and yield (A), and a negative regression between *Meloidogyne* spp. and yield (B) in the 47 plots.

8.4 DISCUSSION

Damage to sett roots by endoparasites impairs the development and growth of shoots. A study conducted in South Africa and West Africa showed that shoot development was suppressed by endoparasites in sett roots (Cadet & Spaul, 1985). In the present study, shoot germination percentage decreased as endoparasite numbers in sett roots increased. Even when there were no nematodes to attack the sett roots, only 60% of the buds germinated (Fig. 5). The non-germination of the other 40 % could be attributed to apical

dominance, damage of buds during cutting of the stalk or destruction by other micro-organisms. Also, the calculated x-value shows that theoretically, it would take 6140 individual endoparasites in a gram of sett roots for germination not to occur at all, i.e. 0% germination. Also, as endoparasite numbers decreased, sett root weights increased because they experienced less damage due to endoparasites. Theoretically, when there were no endoparasites in the roots, about 0.26g of sett roots could grow from each node (Fig. 4). The calculated x-value showed that it would require over 16 million individual nematodes for sett roots weights not to increase at all. The regression line in Fig. 6 shows that even when there were no sett roots, there was 50.3 % germination of shoots. The calculated x-value showed that it would take 1.79 g of sett roots per node for 100 % shoot germination.

The yields (tons/ha) recorded from the different treatments were not significantly different although there was a tendency for plots with high *S. brachyurus* + *H. dihystra* proportions to have higher yields than those dominated by *X. elongatum*. The above average yield, 81.2 tons/ha shown in Fig. 10 in relation to *S. brachyurus* + *H. dihystra* in Fig. 8, is a result of a positive correlation between these species and yield. The regression plotted between *S. brachyurus* + *H. dihystra* percentages in shoot roots and yield showed that when none of these nematodes were present, 18.84 tons/ha of yield was obtained and that maximum yield could be obtained even if 44 % of these two species were present. Plots dominated by endoparasites, e.g. *P. zae*, *Meloidogyne* spp. and *Hoplolaimus* spp. and ectoparasites like *P. minor*, *Tylenchorhynchus* and *Criconea* spp. had below average yield. The regression plotted between *Meloidogyne* spp. and yield showed that theoretically, 30 % yield was obtained when there were no *Meloidogyne* spp. in the roots. Also, the calculated x-value showed that 90.7 % of *Meloidogyne* is what would be required to get no yield at all. *Meloidogyne* spp. cause great damage to plants and that may lead to reduction in yield. The greater yield found in plots whose nematode communities are dominated by *H. dihystra* may not be attributed to the neutralising effect of this species on other nematodes only but also to the nutrients received from the treatments. None of the seven treatments managed to achieve a manipulated nematode community where *H. dihystra* was the dominant ectoparasite because the different treatments showed a lot of variability amongst themselves.

High organic content in soil stimulates microbial activity and increases the activity of beneficial soil microorganisms antagonistic to nematodes (Bridge, 1996). Filter cake, apart from releasing humic acid that is unappealing to nematodes, is also highly nutritious and the plants may have grown quicker because of the readily available nutrients. It may also act as a screen protecting roots from nematode attack. Mixing thume, which has no nutritional value, with filter cake may have boosted the nutritional status of the treatment making it possible for plants to get nutrients easily while thume protected roots from being attacked by nematodes as it has no definite structure and nematodes could not pass through. In trash + filter cake-treated plots, filter cake acted as a source of nutrients while also speeding up the decomposition process for the release of more nutrients and the accumulation of specific compounds that may be

nematicidal (Rodriguez-Kabana, 1986) as some organic amendments may release nematicidal chemicals when decomposing. Aldicarb is a non-fumigant nematicide and its benefits include more rapid development of a full canopy over the inter-rows thereby suppressing weeds resulting in development of roots and efficient uptake of nutrients and water (Cadet & Spaull, 2003). Although furfural has nematicidal properties (Rodriguez-Kabana et al, 1993), plots treated with filter cake and furfural had high nematode numbers in their roots and produced below average yields while plots treated with extra filter cake + furfural had few nematodes and their yield was above average. Furfural is poisonous to developing buds and the extra filter cake protected roots from nematode attacks, neutralised the effect of furfural on buds and supplied the roots with the necessary nutrients.

None of the treatments in this study has successfully managed to manipulate nematode communities for *S. brachyurus* + *H. dihystra*. Even so, greater sugarcane yield was obtained from plots with high abundance and percentages of *S. brachyurus* + *H. dihystra* compared to plots dominated by *X. elongatum*. This confirms what has been observed in West Africa on millet by Villenave & Cadet (1998) and in South Africa on sugarcane by Cadet et al. (2001), where higher yields were obtained in areas with nematode communities dominated by *H. dihystra* than in *X. elongatum* dominated nematode communities.

CHAPTER 9

GENERAL DISCUSSION

Plant parasitic nematodes are found in all sugarcane growing regions (Stirling & Blair, 2000). Some species are highly pathogenic while others are moderately pathogenic. Highly pathogenic nematodes feed on the root hair, the base of lateral roots, or root tips, causing swelling and reduced root growth, e.g. species of *Pratylenchus*, *Meloidogyne*, *Xiphinema* and *Paratrichodorus*. *Pratylenchus* spp. are migratory endoparasites, whose second stage juveniles enter through the root epidermis, enter the cortical cells and move about in the root tissues (Poinar, 1983) parallel to the root axis (Ferraz & Brown, 2002). This species kills the cells in advance before penetration by releasing toxins that diffuse the tissues (Poinar, 1983). Attacked cells usually collapse soon after the nematodes withdraw, leaving behind large cavities within the roots (Ferraz & Brown, 2002). *Meloidogyne* spp. are sedentary endoparasites and spend most of their lives within the cells they parasitise (Poinar, 1983), forming feeding sites. Second stage juvenile larvae of *Meloidogyne* spp. aggregate around the root tip and feed on the epidermal cells in the cell differentiation and elongation regions (Wallace, 1973) as they break the walls and enter the cortex (Poinar, 1983). The pharyngeal gland secretions of the juveniles break down the cell walls (as many as eight) around the nematodes' heads and transform them into giant nurse cells called coenocytium from which they feed until they reach adult stage (Ferraz & Brown, 2002).

Xiphinema and *Paratrichodorus* spp are ectoparasites and feed on plant parts from the outside by inserting their long stylets into the cells (Poinar, 1983). Feeding of *Xiphinema* spp. can last from several hours up to three days (Wallace, 1973), completely destroying the cells within or in the vicinity of vascular tissues in which they feed (Spaull & Cadet, 1990). Parasitism of roots by *Xiphinema* spp. often induces formation of elongated or curled terminal root galls (Ferraz & Brown, 2002). The growth of the meristem cells is retarded and wounds act as entry points for other microorganisms (Poinar, 1983). *Paratrichodorus* spp. are migratory ectoparasites feeding over the entire root surface and can kill the root tip, halting growth and that results in fewer and shorter rootlets.

Moderately pathogenic nematodes feed on epidermal cells, causing lesions, e.g. *Helicotylenchus*, *Scutellonema* and *Criconebella* spp. These ectoparasites "graze" on epidermal cells and because there are many of these cells, the nematodes cause less damage (Spaull, Pers. comm.). Although *Helicotylenchus multicinctus* is the most damaging nematode to banana and plantains worldwide (Ferraz & Brown, 2002), *H. dihystra* caused very little damage to millet roots (Villanave & Cadet, 1999). Also, patches of well grown sugarcane in KwaZulu-Natal, South Africa, were related to soils in which *H. dihystra* was the dominant species in a nematode community (Cadet et al., 2001) but this species showed pathogenicity to sugarcane grown in pots (Rao & Swarup, 1975).

9.1. PATHOGENIC EFFECT OF *H. dihystera* VERSUS *X. elongatum*.

The hypothesis for this study was that a higher percentage of *H. dihystera* than of *X. elongatum* in a nematode community neutralises the parasitic effect of other nematodes, causing less damage to plants and leading to higher yield. Damage caused to sugarcane roots was found not to be a question of the numbers of the different species occurring in a nematode community but rather the proportions in which they occur (Cadet et al. 2001).

Agricultural lands rarely contain monospecific nematode populations but rather various species whose niches overlap (Kraus-Schmidt & Lewis, 1981). According to Norton (1989), plant-parasitic nematodes must compete when their niches overlap since one species may negatively affect or promote the invasion, development and reproduction of another species (Inserra et al., 1984, Kraus-Schmidt & Lewis, 1981). Co-existing species that share a common food source are likely to differ in their reproductive characteristics (Yeates, 1999). Evidence from soybean fields in Michigan suggests that *Heterodera glycines* affected the densities of other plant parasitic nematodes and caused a decline in *Pratylenchus* spp. population densities (Warner et al., 1994). In contrast, *H. dihystera* in millet did not affect the reproduction of *Pratylenchus pseudopratensis* or *Tylenchorhynchus gladiolatus* (Villenave & Cadet, 1998).

In cotton fields in South Carolina, *M. incognita* numbers decreased while *Hoplolaimus columbus* increased probably due to suppression of the former by the latter (Kraus-Schmidt & Lewis, 1981). Interactions between *Meloidogyne javanica* and *Criconebella xenoplax* in South Africa caused premature leaf drop, death of shoots and reduced root systems in peach trees (Hugo & Meyer, 1995). In another study on peach trees in Georgia, *C. xenoplax* was suppressed by *M. incognita* in such a way that no trees died as a result of the antagonism between the two species (Nyczepiet al., 1997). In rice fields in Côte d'Ivoire, *H. dihystera* was undetected before sowing or during the cropping season but was the dominant species at harvest (Coyne et al., 1999). This may have come about as a result of reproduction by the species. Species present before sowing may not necessarily develop to pest status within a single cropping season (Coyne et al., 1999). A negative relationship between root-knot and reniform nematodes in cotton suggested a competitive relationship leading to reduced root-knot densities in heavier soils in which reniform nematodes were better adapted (Kinlock & Sprenkel, 1994).

From the experiment conducted in this study, sugarcane grew taller and produced greater root and leaf biomass in pots with sterilised soil than in pots with naturally infested soil. Soil sterilisation releases nitrogen which can then be easily taken up by plants and in a study conducted by Villenave & Duponnois (1998), *H. dihystera* had no effect on growth of millet when the soil was sterilised. In Australia, when "sick soil" with low organic carbon and few bacteria was sterilised, sugarcane grew better than in non-sterilised soil (Stirling et al. 2001). Thompson (1985) also found that sugarcane yield increased when

planted in sterilised soil as stalks grew longer and heavier. An experiment conducted by Glover (1970) showed that sugarcane grown in sterilised soil grew better from an early age, with dark green leaves compared to the yellowish ones grown on non-sterilised soil. Even below ground, the roots of plants in sterilised soil had a rapid downward growth and branched well. In the present study, sugarcane grown in sterilised soil inoculated with *H. dihystra* grew faster and taller and produced more root and aerial biomass than that grown in sterilised soil inoculated with *X. elongatum*.

Plant-parasitic nematodes do not all have the same effect on plants as some feed shallowly on the root cortex and have less effect on plant productivity, e.g. *Helicotylenchus* spp. (Bernard, 1992), while others feed on the vascular tissues and cause great damage, e.g. *Meloidogyne* spp. Cadet & Spaul (1985) and Cadet et al. (2001) found that areas with good yield were not necessarily a result of good soil conditions or that they were nematode free but rather a result of nematode communities being dominated by the weak pathogen, *H. dihystra*.

The overall growth of sugarcane grown in sterilised soil was significantly different to that of sugarcane grown in natural soil, with aerial and root biomass being greater in sterilised treatments than in naturally-infested treatments. For the natural soils, there was no significant difference in sugarcane growth, aerial and root biomass amongst the treatments although the aerial biomass in the mixed treatment was greater than in high *H. dihystra* and high *X. elongatum* percentage treatments. This may be attributed to *H. dihystra* mitigating the pathogenic effect of *X. elongatum* in the mixed treatment. When *H. dihystra* was inoculated alone on *Acacia holosericea*, it did not reduce its biomass (Villenave & Cadet, 1998). These findings agree with the hypothesis that sugarcane grows better in *H. dihystra* dominated areas than in *X. elongatum*-infested areas, as was witnessed on sugarcane fields in the midlands of KwaZulu-Natal by Cadet et al. (2000).

9.2 NEMATODE COMMUNITY STABILITY AND SPATIAL DISTRIBUTION

The abundance and distribution of nematodes in the soil are influenced by biotic and abiotic soil factors (Dana et al., 2002). A natural plant-parasitic nematode community is generally comprised of different species that interact with each other (Norton, 1989). This was demonstrated on radish whose roots were inoculated with *Meloidogyne incognita* and *M. javanica* (Duncan & Ferris, 1983) where interspecific competition between the two species reduced the amount of damage caused to the host by either species. Competition between *M. incognita* and *Rotylenchulus reniformis* on sweet potato (*Ipomoea batatas*) showed that either of the species is capable of inhibiting the other and becoming dominant (Thomas & Clark, 1983).

Nematode population distribution in the field may reflect competitive interactions (Freckman & Caswell, 1985). When two nematode species occur within the same community, one or both species' populations may be suppressed (Rao & Seshadri, 1981) as the presence of one nematode species may enhance or retard the development of another species on the same host (Griffin, 1983). Direct competition between *Heterodera schachtii* and *Meloidogyne hapla* on sugarbeet, *Beta vulgaris*, led to the growth and development of *H. schachtii* being retarded if *M. hapla* already in the root had not reached the adult stage (Freckman & Caswell, 1985). When *Ditylenchus dipsaci* and *H. schachtii* were inoculated on sugarbeet roots, *D. dipsaci* failed to reproduce and did not affect development or reproduction of *H. schachtii*. Also, interspecific association between endoparasitic nematodes, *Pratylenchus zae* and *Hoplolaimus indicus* on sugarcane showed a negative association between the two species (Sundararaj & Mehta, 1993). As they both depended on roots for food, reproduction and multiplication, a competition for survival occurred between them. When *Meloidogyne graminicola* was inoculated together with *M. incognita* on *Trifolium* spp., *M. graminicola* reproduced at higher rates than *M. incognita* (Windham & Pederson, 1992). *Pratylenchus coffeae* and *Tylenchulus semipenetrans* occurred separately in a citrus grove in Florida but when they were introduced to each other's area, they reduced the populations of the other species (Kaplan & Timmer, 1982).

A certain species may occur in different proportions in different environments and at different times of the year (Ricklefs, 1987). According to Spaull & Cadet (1985), nematode species found on sugarcane ratoon crop grown on similar soil types in West Africa and South Africa showed that *H. dihystra* formed 87 % of the ectoparasite community in Burkina Faso while *X. elongatum* and other ectoparasites formed the remaining percentile. In South Africa 71 % of the ectoparasite community was formed by *Xiphinema* spp. with *H. dihystra* and other ectoparasites forming a smaller percentile.

Generally, plant-parasitic nematodes are patchily distributed in a field (Goodell & Ferris, 1980, McSorley & Parrado, 1982) and this phenomenon may be attributed to differences in soils' physical or chemical properties (Cadet et al., 2001). In Indiana, the soybean nematode pests, *Pratylenchus scribneri* and *Hoplolaimus galeatus* showed a patchy distribution (Alby et al., 1983). A study conducted in sugarcane fields by Cadet et al. (2002) showed that in a termite mound, "isiduli" in Zulu, formed by *Macrotermes natalensis*, *Meloidogyne* was absent and *Xiphinema* less abundant while *Helicotylenchus*, *Pratylenchus* and *Paratrichodorus* occurred in high numbers within the 'isiduli'. Although it is often thought that between-years tillage evens out patchy distribution of plant parasitic nematode populations (Delaville et al., 1996), this is not always the case (Yeates, 1999). A study conducted in Michigan showed that patterning of food web components was maintained despite the homogenising effects of annual soil tillage and monoculture of maize (Robertson & Freckman, 1995).

Studies on spatial patterns of *Meloidogyne* spp. in potato (Wheeler et al., 1994) and tobacco fields (Noe & Campbell, 1985) demonstrated that the population densities change unpredictably in rows more than 10m long. In tobacco fields, spatial correlations for *M. incognita* densities were greater within plant rows than across plant rows. In cotton fields, *M. incognita* varied more over the length of the field than across the width (Wheeler et al., 2000). In the present study, samples were collected at 5m intervals along the transects and high proportions of *X. elongatum* occurred in places with low proportions of *H. dihystra*. This distribution pattern remained stable over the crop cycle and also along the transects implying that the average densities of plant-parasitic nematodes in soil as well as free-living and endoparasitic species in roots remained more-or-less the same at each sampling point and remained stable in their spatial distribution. This pattern of distribution of these two species may be due not only to antagonism between them but also to other factors such as microorganisms assisting or retarding the development of one species. According to Vogel et al. (2002), the possibility exists that a balance between species may be controlled by the composition of the associated bacterial flora. The activity of microbial antagonists may result in the establishment of soil conditions that limit nematode population development for a long period (Westphal & Becker, 2001). In the present study, even though the same crop, sugarcane, has been monocultured for years, the distribution of the different nematode species has not been homogenised, as *X. elongatum* still dominates the first half of the transects while *H. dihystra* dominates the second half.

As the distribution of plant roots impacts on that of plant-parasitic nematodes since they are part of the host plant (Noe & Campbell, 1985), uniformity or non-uniformity in growth of the host may be responsible for the patchy nematode distribution. In chapter 5, high percentages of *X. elongatum* occurred in areas with low percentages of *H. dihystra* and vice versa along a homogenous sugarcane row and they remained the same at corresponding sampling points in adjacent rows. This conforms to the view that nematodes occur in patches in an agricultural field. The conclusion therefore is that the hypothesis that monocropping for decades evens out the patchy distribution of nematodes did not apply in this study and that if the host plant was not responsible for the observed difference in nematode distribution, the environment must be responsible. Either the physical or chemical properties of the soil or microorganisms such as bacteria or fungi found in it were responsible for this observed difference in species distribution.

9.3. USE OF ABIOTIC SOIL FACTORS IN NEMATODE COMMUNITY MANAGEMENT

The soil environment affects nematode population growth and species' survival through the impact of its biological, physical and physiological components (Cuc & Prot, 1992; Wallace et al., 1993). These soil properties also control water, air and nutrient movement through the soil (Poulsen et al., 2001). According to Warren & Linit (1992), within-tree moisture conditions influenced the dispersion of the pinewood nematode *Bursaphelenchus xylophilus*. Dwinell (1986) reported that a decrease in the moisture percentage of wood chips caused the *B. xylophilus* densities to decline.

Chemicals found in soil may also impact on nematode populations. In rice fields in Senegal, low nematode populations were attributed to the nematicidal properties of soluble sulphides found in soil (Jacq & Fortuner, 1979). Another study done in Senegal showed that *Hirschmanniella oryzae*, *H. spinicaudata* and *Tylenchorhynchus mashhoodi* were killed by soluble sulphides (Fortuner & Jacq, 1976).

Some soils or regions are unsuitable for certain nematode species and thus have fewer nematode individuals or communities (Cadet & Thioulouse, 1998). Some soils are termed “resistant” as crop damage is less than would be expected from the nematode population in them e.g. roots of sugarcane grown in heavier soils have less damage than those of sugarcane planted in sandy soil (Spaull & Cadet, 1990). This is due to slower invasion of roots by nematodes and not because of fewer nematodes in those soils (Cadet et al., 1982). Root-knot nematode infestations and associated crop damage are greater in sandy soils than in clay or heavy soils (Barker et al., 1981, Shane & Barker, 1986). Contrary to the general view, nematode populations in heavy soils in Queensland were sometimes as high as they were in sands (Stirling et al., 1996). *Pratylenchus zae* and *Meloidogyne* spp. occurred in significant densities and their distribution were not related to clay content as they were more commonly found in clay loam and heavy clay soils than in sand (Stirling & Blair, 2001). In South Africa, *H. dihystra*, *P. zae* and *Paratrichodorus minor* occurred in higher numbers in termite mounds with a greater clay content than the surrounding areas (Cadet et al., 2002).

Soil texture too plays an important role in the relationship between soil and nematode species (Cadet & Thioulouse, 1998) as it influences their abundance and distribution (Norton, 1989, Upadhyay et al., 1972, Yeates & Bird, 1994)). It can influence nematode populations by allowing or restricting movement of nematodes towards roots or mates (Norton, 1979, Hassink et al., 1993). It also interferes with the uniformity of distribution of nematodes by influencing the abundance and distribution of many species (Cadet et al., 1994). The influence of soil texture may however vary with nematode species (Barker & Weeks, 1991). *Heterodera schachtii* on sugarbeet reproduced better in a silt loam soil than in sandy loam but the reverse was true for *M. hapla* (Santo & Bolander, 1979). *Ditylenchus dipsaci* reproduced well in soils with a high (73-100 %) clay content, *M. hapla* reproduced best in sandy soil (82-91%) while *Rotylenchus robustus* reproduced well in all soils (Upadhyay et al., 1972). *Pratylenchus hexincisus* reproduced rapidly on corn in Iowa (Zirakparvar et al., 1980). Most ectoparasitic nematodes are favoured by coarse-textured soils (Norton, 1979).

Many soil properties are spatially correlated, i.e. areas closer to each other are likely to have similar soil characteristics than more distant areas (Burrough, 1993). According to Wagenet & Jurinack, 1978, changes in soil texture, mineral content and salts may determine spatial differences in the physical properties of soils. In the present study, the individual soil elements and particle sizes showed homogeneity in their

spatial distribution amongst the five transects, although their occurrence in relation to one another was different. Although silt, clay, fine and medium sand showed homogeneity in their distribution along the five transects, clay occurred in areas with low medium sand content and vice versa while silt was found in areas with clay soil. This particle size gradient along the five transects implies that silt and clay were less common in areas with fine and medium sand contents. The soil elements also showed heterogeneity in their occurrence along the five transects, with high Al levels occurring at sampling points with low Mg, Na, Mn, Zn and K levels.

Nematode densities represent a response to soil environment (Noe & Barker, 1985, Hassink et al. 1993) and variation in physical and chemical soil properties may have different effects on nematode populations. Differences in pH, (Ferris et al., 1971), organic matter (Norton & Schmitt, 1974), soil texture and moisture (Schmitt, 1973) have been related to nematode occurrence. Some nematode species have an affinity for certain soil elements as was shown in a large scale survey in KwaZulu-Natal where high numbers of *Pratylenchus zaei*, *H. dihystra* and *Paratrichodorus minor* were inversely related to pH but not to Fe (Spaull & Cadet, 2001). In the same study, *Meloidogyne* spp. were inversely related to Fe but positively related to pH. Noe & Barker (1985) found that the elements like nitrogen, sodium, magnesium and copper may correlate with nematode community structure. High concentrations of sodium cations may improve the ability of the plant parasitic nematode to find a host while high copper concentrations had a negative correlation to nematode density, implying that this cation was toxic to plant-parasitic nematodes. *Pratylenchus coffeae* was linked to pH, sodium, calcium and magnesium in soils planted with tomato and yam in Martinique (Cadet & Thioulouse, 1998).

As nematodes use chemo-reception to locate food and mates, changing the balance of ions in the soil solution can affect the way nematodes perceive root exudates such that they may be unable to locate food or mates and if they cannot find mates, they cannot reproduce (Spaull & Cadet, 2001). Soil particles cannot be manipulated while soil elements can be changed to achieve the desired result, e.g. by increasing or decreasing levels of certain soil elements that have an affinity with certain desired nematode species, the nematode community can be manipulated in favour of the desired species composition.

High proportions of *H. dihystra* were found in sampling points with high levels of calcium, magnesium, aluminium, manganese, zinc, iron, silt, clay while *X. elongatum* had a negative correlation with these soil elements. High *X. elongatum* percentages occurred in soil with high, medium and coarse sand content. Also, this species showed a weak positive correlation with phosphorus and sulphur. The relationship observed between the soil elements and certain nematode species does however not imply a cause and effect relationship, as other factors may be responsible, e.g. soil microorganisms. Wallace (1973) and Norton (1978) both suggested that soil factors other than soil type were important in determining the distribution of nematodes.

9.4. IMPACT OF NEMATODES ON SOIL AND LEAF RELATIONSHIPS.

Efficiency in nutrient uptake differs amongst plants in a field, depending on availability and efficiency of their root systems. Plant-parasitic nematodes have a pathogenic effect on plants and by damaging plant roots, they interfere with the efficiency of the roots in taking up nutrients from the soil (Dana et al., 2002). In the present study, the growth of sugarcane was homogenous and therefore root growth was more-or-less the same. Leaves from the first part of the transects had high N levels while leaves on the second part had high Ca levels.

Iron uptake and transport within the plant is highly affected by environmental factors (Al-Mustafa et al., 2001). Available phosphorus in soil is rarely enough for optimal plant growth and in many regions of the world it is the main factor controlling crop growth. Phosphorus is second only to nitrogen in limiting crop production (Miyasaka & Habte, 2001) followed by potassium (Adediran & Banjoko, 1995). In tomatoes planted in saline medium, the addition of phosphorus resulted in high phosphorus levels (Satti et al., 1995), low levels of sodium and high levels of potassium in leaves (Awad et al., 1990). In this study, physical soil properties and leaf elements showed a strong correlation amongst themselves. Clay and medium sand had a positive correlation with leaf Fe. Sugarcane plants with high leaf Fe levels grew in soils with high clay and medium sand contents. Leaf Ca had a strong positive correlation with silt and a negative one with coarse sand, implying that plants grown in soils with high silt content would have high levels of calcium in their leaves while those grown in high coarse sandy soil would have low leaf Ca levels. Plants with high nitrogen levels in their leaves grew in areas with high levels of coarse sand and the reverse was true for those that grew in silt soils.

The level of a certain element in the soil usually correlates with the level of that element in the leaves of a plant grown in that soil. But within the plant, some elements are antagonistic or synergistic to each other. From Mulder's chart, P and Mg show synergism between them. High levels of P and Mg in soil led to high levels of these elements in the leaves sampled in areas where these elements occurred in abundance. Magnesium and Ca as well as Mn and Ca show antagonism to each other. This was also seen in the present study with soil Ca showing a negative correlation with Mg and Mn in leaves. High levels of Fe in soil, and therefore in roots as well, led to low levels of leaf Fe as this element was negatively correlated in the two parts of the sugarcane.

A relationship between the distribution of certain leaf elements and nematode species was therefore identified. The varying levels of elements found in the leaves may be due to the nematode species found in the soil where the plants grow. High proportions of *X. elongatum* were found at sampling points with high nitrogen levels in leaves while the opposite was true for *H. dihystra*. Plants with high levels of Ca in their leaves grew in areas with high proportions of *H. dihystra* and low proportions of *X. elongatum*. In a study

conducted by Franzen & Peck (1995), sugarbeet plants were found to have a Ca deficiency despite adequate calcium levels in nutrient solution. The relationship observed between the leaf elements and certain nematode species may imply a cause and effect relationship, although other factors may also play a role, e.g. other soil microorganisms, root efficiency and element availability. The mutualistic symbiosis between arbuscular mycorrhizal fungi and maize (*Zea mays*) stimulated phosphorus absorption by the plant (McGonigle & Miller, 1993). A study conducted on peach trees in Texas showed that levels of zinc and sulphur in leaves increased linearly with elevated zinc rates in soil while copper levels in soil caused an increase in leaf nitrogen (Powell et al., 1995).

A relationship between nematodes, soil elements and leaf elements and that the distribution of one of them affects the distribution of the others was shown. *Xiphinema elongatum* dominated the nematode communities found over the first half of the transects while *H. dihystrera* dominated over the second half. High proportions of *X. elongatum* were found in areas with high P, medium and coarse sand levels while high *H. dihystrera* proportions occurred in areas with high Mg, Ca, silt, clay and fine sand levels. Leaves with high levels of Ca, Zn and Cu were found in areas with high proportions of *H. dihystrera* and in soil with high silt and clay contents while leaves with high levels of N were found in areas with high proportions of *X. elongatum*. Plants with high leaf P levels grew in soil with high coarse sand content, the same soil where high proportions of *X. elongatum* were found.

The relationships observed in this study demonstrate that nematode species, soil physical properties and leaf elements are inter-linked and have an influence on each other. This implies that soil physical properties affect the occurrence, abundance and distribution of nematodes that in turn affect the roots' efficiency in the uptake of soil elements and therefore their availability to plants.

9.5. ALTERNATIVE METHODS TO CONTROL NEMATODES THROUGH ORGANIC AMENDMENTS.

Soil is a non-renewable resource (Widmer et al., 2002) and many agricultural soils around the world are deficient in one or more of the essential nutrients for healthy and productive plant growth (Baligar et al., 2001). Both organic and inorganic amendments have been, for centuries, added to soil to improve its fertility, stimulate microbial activity, increase crop yield and manage plant-parasitic nematodes (Rodriguez-Kabana, 1986, Bridge, 1996). Management systems that use organic amendments such as crop residue or waste material can increase the soil carbon level and that may lead to soil quality improvement (Lal et al., 1999, Fortuna et al., 2003). Thus organic wastes like manure and crop residues are commonly used as soil amendments and plant nutrient sources in agricultural soils (Hoitink & Boehm, 1999, Rogers et al., 2001). Organic matter in soil promotes microbial activities and nutrient availability and its deficiency leads to reduction in crop productivity (Wahid et al., 1998). Microbial decomposition of plant residue has a large influence on the composition of soil organic matter (Ding et al., 2002) which is a key factor determining

soil quality (Doran & Parkin, 1994) because it influences the physical and biological properties of soil and availability of plant nutrients (Bessam & Mrabet, 2003). Organic matter also reduces soil compactibility (Zhang et al., 1997) as soils without it become hard and compact (Haynes, 1997). It improves water-holding capacity (Hamblin & Davies, 1977, Francioso et al., 2000, Wander & Yand, 2000) and may increase or decrease pH depending on the nature of the organic amendment (Pocknee & Summer, 1997). Significant reductions in soil pH and sodicity and high yields were recorded when farmyard manure was added to wheat and rice fields (Minhas et al., 1995).

To date, plant-parasitic nematodes have been controlled through use of nematicides and fumigants but because these chemicals have deleterious effects on human health and on the environment, they are being withdrawn from the market (Oka & Yermiyahu, 2002). The phasing out of methyl bromide in recent years has made nematode control more difficult (Noling & Becker, 1994). Several non-chemical alternatives for plant-parasitic nematode management are however available (McSorley & Duncan, 1995), e.g. crop rotation, resistant varieties and organic amendments (Oka & Yermiyahu, 2002). In Nigeria, due to high cost of pesticides and the potential hazards they pose to the environment, humans and livestock, small- and medium-scale farmers use traditional and organic methods of pest and disease control (Poswal & Akpa, 1991, Bridge, 1996). These methods include organic amendments, vegetable oils, ash and neem (*Azadirachta indica*) extracts. Ash, neem, palm and groundnut seed oils are used to control insects from damaging cowpeas, pearl millet and sorghum in storage while neem powder is used for pest control in the field. In South Africa, animal manure is used as a source of plant nutrients by small-scale farmers (Materechera et al., 2000). Cow dung and poultry manure have succeeded in controlling *M. incognita* on tomato plants (Chindo & Khan, 1990, Poswal & Akpa, 1991).

Composts are used as soil amendments to provide nutrients and organic matter and to improve soil structure (Oka & Yermiyahu, 2002). They are generally prepared from waste products such as tree bark, animal and plant waste, municipal solid waste and sewage sludge and are widely used to control diseases caused by soil-borne plant pathogens (Huang & Huang, 1993, Michel & Mew, 1998). Plant-parasitic nematodes have been successfully controlled by the use of organic amendments in many parts of the world especially areas with high nitrogen content soils as they have been reported to have nematicidal effects (Rodriguez-Kabana, 1986). Mian & Rodriguez-Kabana (1982) discovered that chicken litter, with its high nitrogen content, can suppress root gallings caused by *Meloidogyne* spp. When enough nitrogen and carbon were available, microbial activity increased and resulted in the creation of a nematode-antagonistic environment. Microbial activity may result in production of nematicidal compounds and ammoniacal nitrogen. Approximately 95 % of soil nitrogen and about 50 % of soil phosphorus is present in soil organic matter. The most effective materials of this type are composts, oil meals and cakes, animal and plant manure (Rodriguez-Kabana, 1986).

Some soil amendments suppress bacterial wilt, a soil-borne disease that affects a wide range of staple and cash crops, e.g. tomato (*Lycopersicon esculentum*) and banana (*Musa spp.*) and is caused by *Pseudomonas solanacearum*. Compost made from tree bark showed suppressive effects on plant pathogenic fungi that may cause root rot, *Rhizoctania solani* and *Pythium ultimum* (Erhart et al., 1999). Evidence from other cropping systems suggests that soil amendments might suppress *Pythium* root rot in sugarcane (Dissanayake & Hoy, 1999). Soil amendments increase plant vigour, thereby enhancing tolerance to nematode and disease attacks (Poswal & Akpa, 1991).

Studies in application of non-traditional organic wastes such as food-processing residuals and yard wastes have shown benefits such as plant nutrients and improved soil structure (Rogers et al, 2001). Disposing of treated sewage sludge is becoming more difficult because of population and industrial growth (Turner et al., 1991). Farmers, municipalities and industries realise that application of these wastes on crop fields is a cost-effective means of disposal (Heckman & Kluchinski, 1996). In Durban, an experiment was done where sewage sludge was used as a soil amendment in sugarcane fields. The sludge provided nitrogen and phosphorus to sugarcane crops over a long period and substantially increased yield (Turner et al., 1991). Composted sewage sludge showed some activity against root-knot nematodes on potato plants (*Solanum tuberosum*) (LaMondia et al., 1999) and also suppressed *Pythium* damping-off in cucumber and cotton (Lewis et al., 1992). However, a study conducted in Germany showed an increase in plant-parasitic nematode numbers after sewage sludge application (Weiss & Larink, 1991) but this was attributed to higher plant biomass and therefore greater root biomass and more food. Also, sludge from paper mills is used as soil amendments for various crops such as grasses and legumes (Zhang et al., 1993). Pulp and paper mill sludge contributes to organic matter content, and may change the physical and chemical properties of soil (Vagstad et al., 2001). Also, in KwaZulu-Natal, bagasse is used as a filler in paper manufacture and the waste produced is combined with other waste material produced during the process. This mixture is then dewatered to form thume which is then dumped on nearby land. In KwaZulu-Natal, sugarcane fields treated with waste from a paper mill had a growth rate three times or more than non-treated fields (Hughes & Girdlestone, 2001).

In sugarcane fields, accumulation of soil organic matter and improved soil health can be achieved through green cane harvesting (Graham et al., 1999). Trash contains large quantities of dry matter and nutrients, especially nitrogen and potassium that are released when it decomposes (Thorburn et al., 1999, Mitchell et al., 2000). Pre- or post-harvest burning of sugarcane makes the system lose 80-95% of its dry matter and nitrogen. In India, chopped and unchopped trash increased sugarcane yield from 141 t/ha to 154 t/ha and 152 t/ha respectively (Jadhav, 1996). The use of sudangrass as green manure suppressed *M. hapla* and reduced the damage it caused on lettuce in organic soil (Viaene & Abawi, 1998).

Damage to sett roots by endoparasites impairs the development and growth of aerial shoots. Root-feeding nematodes thus cause great loss in sugarcane yield in South Africa (Vogel et al., 2002). In studies conducted in South Africa and West Africa, very large numbers of endoparasitic nematodes were recorded in sett roots of sugarcane in both localities (Cadet & Spaul, 1985) and as a result, shoot development was suppressed. A study on *M. incognita* management on *Hyoscyamus muticus* using chemicals and soil amendments showed that the suppressive effect of the treatments on nematode populations in roots and soil was highly significant.

In chapter 9, organic amendments were localised in the furrows and not scattered all over the field, the idea being to “sandwich” and protect sugarcane roots from nematode attack. In sett roots, control and filter cake + furfural-treated plots had the highest numbers of endoparasites while aldicarb and thume + filter cake-treated plots had the lowest numbers. In shoot roots, control plots had highest endoparasite numbers followed by filter cake-, filter cake + furfural- and aldicarb-treated plots which showed no significant differences amongst themselves. High amounts of filter cake (referred to as extra filter cake) extra filter cake + furfural-, thume + filter cake- and trash + filter cake-treated plots had the lowest endoparasite numbers and also had no significant difference amongst them. Aldicarb is a nematicide and 2-furfuraldehyde, the active ingredient of furfural, is a natural fumigant produced commercially from sugarcane bagasse. It also has insecticidal, fungicidal and nematicidal properties (Daneel & de Jager, 1996). Filter cake succeeded in reducing nematode numbers in sett roots of aldicarb- and extra-filter cake + furfural-treated plots. Higher endoparasite numbers in sett roots of filter cake- and trash + filter cake-treated plots than in thume + filter cake-treated sett roots may have been due to thume preventing nematodes from passing through from the soil to the roots as it does not have a structure. Shoot roots of both trash + filter cake- and thume + filter cake-treated plots had few endoparasites. This may be attributed to the humic acid released by filter cake as it decomposed being inimical to nematodes.

High percentages of *H. dihystra* were found in plots with filter cake in their treatments while high *X. elongatum* percentages were in the control plots. As an organic amendment, filter cake might have promoted establishment of microorganisms that favour the development and success of *H. dihystra* rather than *X. elongatum*. More studies need to be conducted to determine why *X. elongatum* proportions were lower than those of *H. dihystra* in treatments with filter cake. Additionally there was no significant difference in yields obtained from the different treatments. The best yield was found in *H. dihystra*-dominated plots. Even in the “isiduli”, where a variety of nematode species was found, the yield was higher than in surrounding areas, corresponding to 89 tons cane/ha in the “isiduli” and 15 tons cane/ha in other areas (Cadet et al., 2002). This was not due to the absence or fewer plant parasitic nematodes but because the “isiduli” promoted the numbers of *H. dihystra*, a species already found to have an association with better sugarcane growth. Of the organic amendments, thume + filter cake gave the best yields

followed by extra filter cake + furfural, filter cake, filter cake + furfural and trash + filter cake. Filter cake is rich in organic nutrients so that, during decomposition, it might have helped the sugarcane to grow.

In the present study, areas with high percentages of *H. dihystra* had low percentages of *X. elongatum* and vice versa. According to Villenave & Cadet (1998), *H. dihystra* neutralised the pathogenicity of other nematodes in millet and circumstantial evidence from West Africa suggests that the same may be true for sugarcane (Cadet et al., 2001). In another study conducted by Villenave & Cadet (1999), millet root biomass was higher in soils with monospecific populations of *H. dihystra* than in soils with other monospecific nematode populations. Also, patches of well-grown sugarcane in KwaZulu-Natal, South Africa were observed in soils with nematode communities dominated by *H. dihystra*. Although none of the treatments managed to manipulate the nematode communities for the less pathogenic species, the plots treated with filter cake and thyme had high percentages of *H. dihystra* and these *H. dihystra*-dominated plots produced higher yields than *X. elongatum* dominated plots.

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