NEMATODE ABUNDANCE AND DIVERSITY IN TWO SUGARCANE FIELDS AT SECTION 10, HIPPO VALLEY ESTATES

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Abstract

There has been a decline in sugarcane yields in fields 1051 and 1053 at Hippo Valley Estates section 10, from an average of 116 t/ha in 1999 to 84 t/ha in 2007. Several factors have contributed to this decline, among which may be plant parasitic nematodes. The aim of this study was to identify plant parasitic nematodes associated with sugarcane in fields 1051 and 1053. Samples of soil and sugarcane roots were collected from the two fields. Samples were collected from areas with stunted cane and areas that had healthy cane. Soils were collected from the cane root zone and from areas 0.75 metres away from the cane row. Nematodes were extracted from soil and root samples using the Baermann technique. Nematodes were identified to genus level and the number of nematodes per 100 grams of soil and one gram of root material were determined. Seventeen genera of ectoparasitic and endoparasitic plant nematodes were identified from soils and sugarcane roots. Most nematodes were identified in soils collected from sugarcane root zones. Soils collected from root zones of stunted cane had significantly more nematodes compared to soils from root zones of healthy cane. *Scutellonema* sp., *Pratylenchus* sp. and *Tylenchorhynchus* sp. were present in >90% of the soil samples collected from sugarcane root zones. *Scutellonema* sp. and *Criconemella* sp. were identified in >90% soil samples collected from 0.75 metres from the cane row. *Rotylenchulus* sp., *Pratylenchus* sp. and *Meloidogyne* sp. were collected from sugarcane roots. These results are discussed in relation to the potential of plant parasitic nematodes in reducing sugarcane yields.

Keywords: plant parasitic nematodes, sugarcane, abundance, diversity

Introduction

Plant feeding nematodes are important in agriculture because they inhibit root growth and eventually overall plant development. This results in poor crop performance and sometimes crop failure. Crop conditions such as lack of vigour, stunting, reduced yields and poor quality product are indicative of water and nutrient deficiencies, which in turn indicate dysfunctioning root systems and possible nematode involvement (Anon, 2000).

Plant parasitic nematodes have been reported in sugarcane since the late 1800s (Williams, 1969; Prasad, 1972). Several nematode species, particularly those belonging to the orders Tylenchida and Dorylaimida have been associated with sugarcane roots (Williams, 1969; Heyns, 1971; Prasad, 1972; Anon., 2000). In South Africa, the roots of sugarcane are fed upon by more than 90 species of nematodes (Spaull and Cadet, 2003). The most common and those causing the most damage are *Meloidogyne javanica*, *Pratylenchus zeae*, *Xiphinema elongatum* and *Paratrichodorus* sp. In Zimbabwe, plant parasitic nematodes were found associated with sugarcane (Martin, 1962), and the most common nematodes were *Pratylenchus* sp., *Trichodorus* sp., *Tylenchorhynchus* sp. and *Scutellonema* sp.

Monocropping over many years, as is common in sugarcane agriculture, enhances the horizontal spread of common nematodes and leads to more uniform distribution (Delaville *et al*., 1996, Cadet *et al*., 2002a). However, it has also been shown that soil texture interferes with the uniform spread of nematodes, influencing their abundance and distribution (Cadet *et al*., 2002a, Chirchir *et al*., 2008). Species of *Meloidogyne* sp. are frequently more numerous and more pathogenic in light textured soils (Spaull *et al*., 2003), giving rise to greater symptom expression in the sandier areas within a field.

Fields 1051 and 1053 at Section 10, Hippo Valley Estates (HVE) were opened for sugarcane cultivation in 1998 and N14 was planted. Good crop yields, usually over 100 tonnes cane/ha, were realised up to 2003, after which yields started to decline (Table 1). Patches of poor cane growth were noticed and soil tests showed differences in soil texture (Table 2), with some portions of the fields having sandy soils. It was suspected that nematodes could be contributing to the poor cane growth in the fields. Since there was no information on occurrence of plant parasitic nematodes in these fields, the aim of this study was to determine occurrence, abundance and diversity of nematodes in fields 1051 and 1053.

Table 1. Sugarcane yield (tonnes cane/ha) over a nine-year period, in fields 1051 and 1053 at Section 10, Hippo Valley Estates in Zimbabwe.

Year	Tonnes cane per hectare	
	Field 1051	Field 1053
1999	108.51	122.82
2000	87.59	101.20
2001	104.68	99.42
2002	124.40	100.21
2003	119.76	109.56
2004	89.36	94.72
2005	90.07	77.49
2006	98.65	97.02
2007	75.95	91.21

Table 2. Part of a soil analysis report for soil samples collected from field 1051 at Section 10, Hippo Valley Estates, Zimbabwe.

Sample No.	Cane growth	Mechanical analysis (%)				OM (%)	pH
		Clay	Silt	Sand	Texture		
1	Stunted	4	4	92	S	1.04	6.51
2	Stunted	4	6	90	S	0.65	6.86
3	Mixed	4	8	88	LS	0.65	6.95
4	Mixed	8	10	82	LS	1.42	6.91
5	Stunted	2	6	92	S	0.78	6.95
6	Stunted	2	6	92	S	0.78	7.04
7	Stunted	4	6	90	S	0.83	6.81
8	Stunted	2	6	92	S	0.52	6.96
9	Healthy	10	6	84	SaL	1.55	6.66
10	Healthy	6	6	88	LS	1.16	6.88

S = sandy, LS = loamy sand, SaL = sandy loam

Materials and Methods

Collection of soil and plant root samples

Moist soil and root samples were collected from fields 1051 and 1053, on 14 and 15 January 2005, using guidelines outlined in a manual for nematology (Anon, 2000). The N14 sugarcane crop was five months old and in the sixth ratoon. A part of field 1051 was divided into 10 one-hectare blocks (A to J) (Figure 1a), while part of field 1053 was divided into two one-hectare blocks (K and L) (Figure 1b). Each hectare was subdivided into four equal quadrants (A1, A2, A3, A4; …K1, K2, K3, K4) (Figure 1c). The fields were further stratified into areas with healthy, vigorously growing sugarcane (healthy cane) and areas with stunted, unhealthy looking cane (stunted cane) (shaded areas in Figure 1a, b).

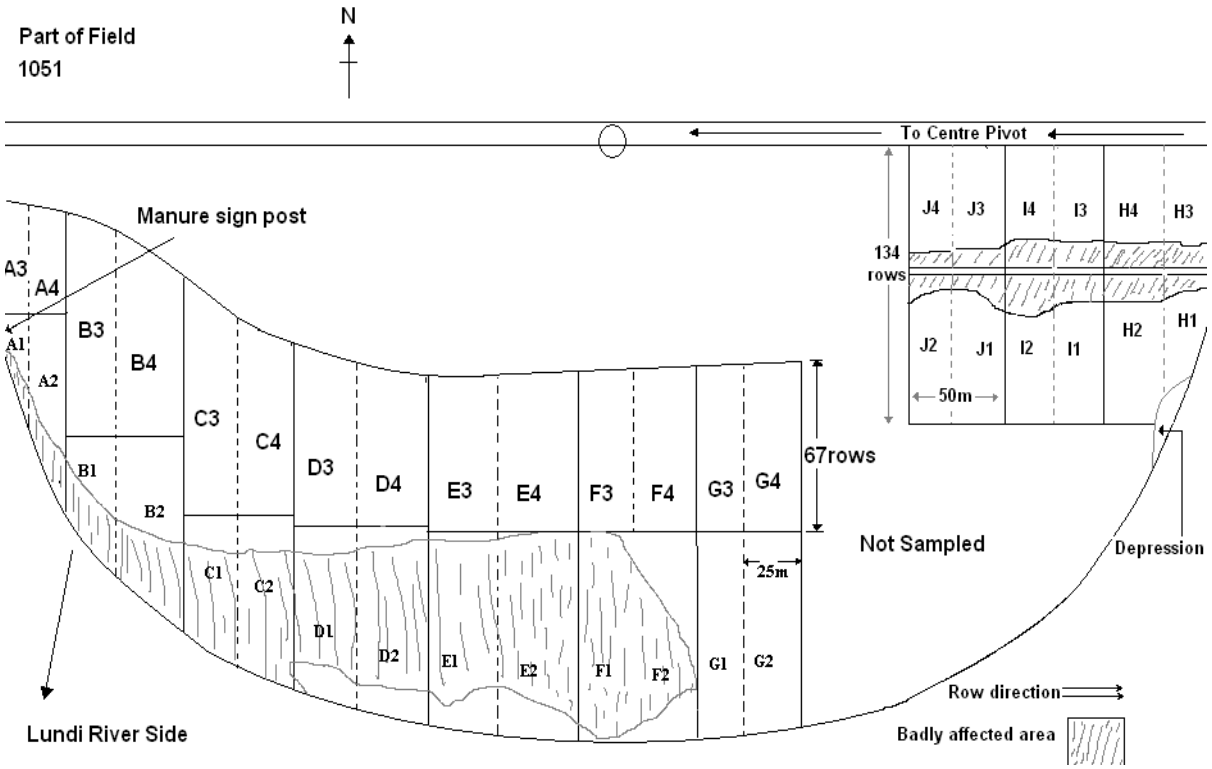

Figure 1a. Part of field 1051 at Section 10 Hippo Valley Estate, showing one-hectare blocks A-J, which were further subdivided into ¼ hectare plots (1-4). Areas with stunted cane are shaded.

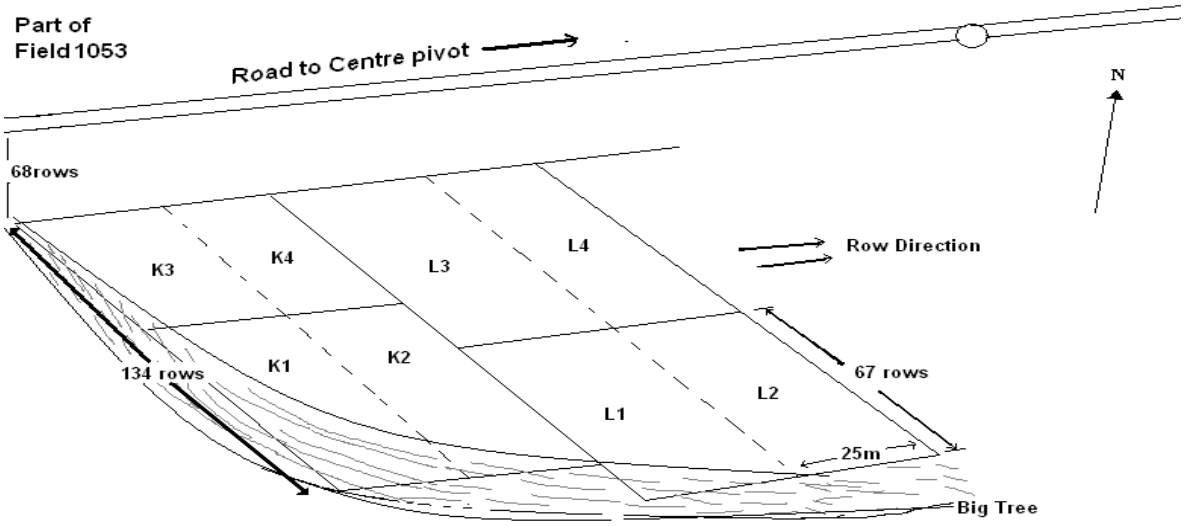

Figure 1b. Part of field 1053 at Section 10, Hippo Valley Estates, showing one-hectare blocks K and L, which were further subdivided into ¼ hectare plots (1-4). Areas with stunted cane are shaded.

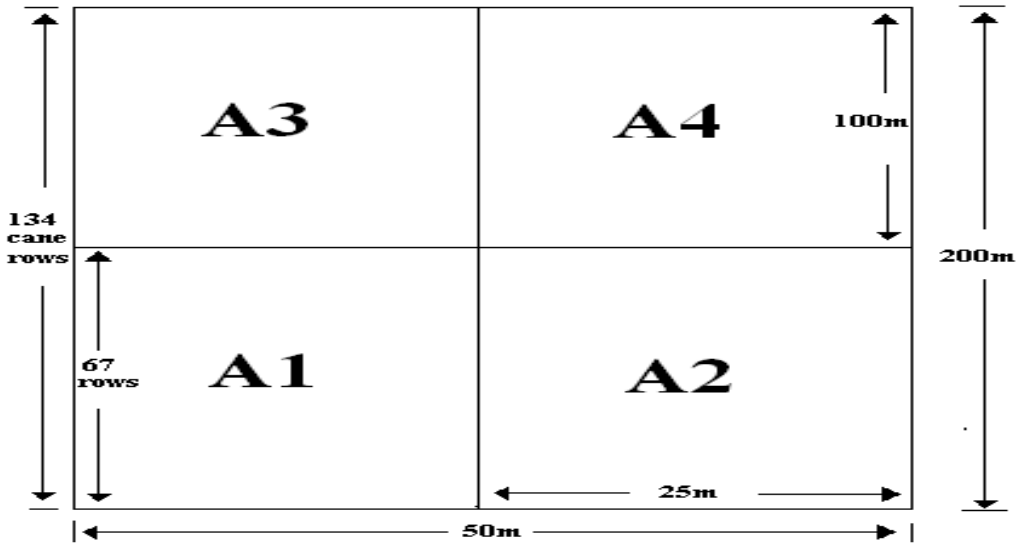

Figure 1c. Relative sizes of ¼ hectare plots (quadrants) in a one-hectare block. The letter (A) is the block label; the number (1-4) is the quadrant number. Each quadrant had 67 cane rows, spaced at 1.5 m (equivalent to 100 m) and was 25 m long.

An Edelman soil auger was used to collect soil samples (500 g per sample) and the soil was collected from the top 30 cm. In the field, samples were kept in the shade in cooler boxes. At the Experiment Station, samples were kept in the cold room at 4ºC, until they were taken to the Nematology Section, Plant Protection Research Institute, Agricultural Research and Extension Services (AREX) at Harare, for analysis.

From each hectare, two sugarcane plants (one healthy and one stunted) were randomly selected and dug out using a hoe. The uprooted sugarcane plants included roots and approximately two kilograms of soil around the roots. Both plant and soil samples were labelled. Later in the laboratory these samples were separated into sugarcane roots and soils from the root zones of the sugarcane plants.

From each quadrant, three composite soil samples were systematically collected 0.75 m away from the sugarcane row (centre of inter-row). Each composite soil sample (1.5 kg) was made up of three sub-samples (500 g each sub-sample) that were collected from the same level in the quadrant as illustrated in the grid pattern in Figure 2. Although three sub samples were collected per level in a quadrant, these were not mixed, but retained as separate sub-samples, and tied together (in threes) to form the composite sample.

Stratified sampling (Levels 1 to 3)
From Figures 1a and 1b, it was noted that most of hectare blocks covered areas with both healthy and stunted cane. Sampling at the different levels in the quadrants (Figure 2) made it possible not to mix soil samples from distinct areas with healthy growing cane and areas with stunted cane.

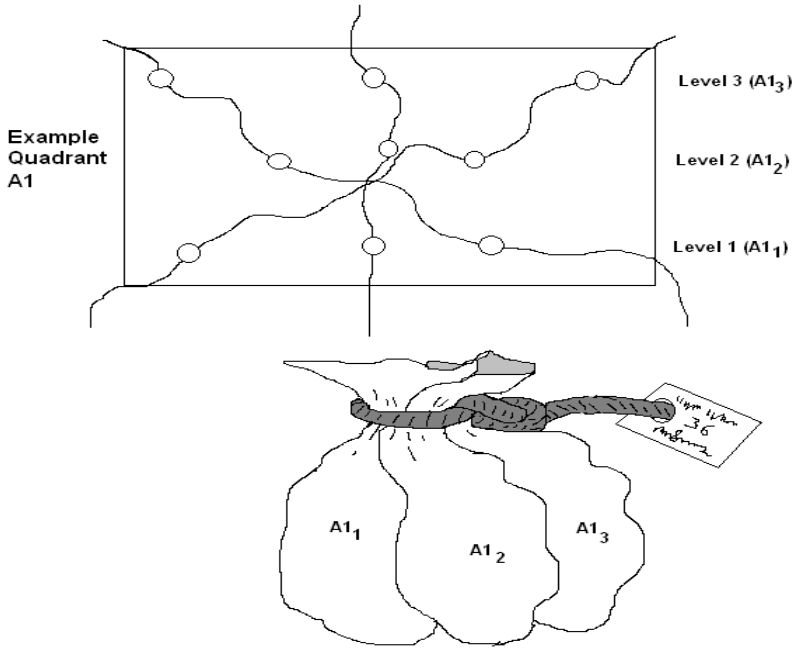

Figure 2. Diagrammatic representation of stratified sampling in each of the quadrants. Soil samples were collected randomly along the paths shown, and sample bags from the same level were grouped together without mixing as illustrated. Notation: the letter (A) is the block label; the first number (1-4) is the quadrant number; the second subscript number (1-3) is the stratified sampling level.

Nematode extraction (Anon, 2000)
Twenty-four sugarcane plants (12 healthy, 12 stunted), each plant with ±2 kg soil, were sent to AREX for analysis. In addition, 144 composite soil samples from the inter-rows were also sent (12 composite soil samples collected per hectare from the 12 hectares). Soil samples were gently but thoroughly mixed by hand, avoiding excessive handling to prevent mechanical damage to the nematodes. The soil was sub-sampled by the coning and quartering method, until the required 100 g was achieved.

The Baermann technique was used to extract nematodes from 100 g of soil and 50 g of root tissues. The Baermann tray method extracts only live, active nematodes from soil and plant material, into a clear suspension.

Soil: One hundred grams of soil were spread on a circle of single-ply paper towel supported on a coarse-meshed plastic screen standing in a plastic container. Water was added to the container until the soil was thoroughly wet but not immersed. The container was covered to reduce evaporation of the water and the set-up was left to stand for at least 24 h undisturbed. The soil was then removed and discarded and the nematode suspension poured from the container into a 250 ml beaker.

Plant: The plant roots were rinsed free of soil and cut into very small pieces (1-2 mm long). Fifty grams of the root material was spread evenly on filter paper and the subsequent processes were as described for the soil extraction.

Counting nematodes

With the nematode suspension in a 250 ml beaker, the specimens were allowed to settle for about one hour. The volume of the suspension was adjusted to 100 ml by adding or withdrawing water. A 10 ml sub-sample of the suspension was withdrawn with a graduated 25 ml pipette and transferred to a counting dish. Under a transmission-dissecting microscope, all plant parasitic nematodes were identified to genus level and the number of specimens of each genus counted using a counter. The sub-sample was returned to the nematode suspension and the counting procedure repeated two more times. All counts were multiplied by 10, and the average number of specimens in each genus calculated.

Data analysis

Nematodes were identified to genus level and the frequency of occurrence of the different genera compared among the four habitats (healthy cane root zone, stunted cane root zone, 0.75 m from the healthy cane line and 0.75 m from the stunted cane line).

Nematode relative abundance of each genus was measured as the proportional number of that genus relative to the total number of nematodes per 100 g of soil (for soil samples) or as the proportional number of that genus relative to total number of nematodes per one gram of plant roots (for root samples).

Relative abundance/richness pi = n/N;
where n is number of individuals of a particular genus;
N is total number of all nematodes counted per habitat.

The Shannon-Wiener index (H'), which is the commonly used index of diversity, was used to calculate the nematode diversity in the habitats (Harrison *et al*., 2004).

$$H' = - \sum p_i \log_e p_i$$

where p_i is the proportion of the *i*th species in the total sample. In practice any log base may be used, and any unit in addition to species may be used in the formula. In this study natural log to base *e* was used, and the unit used was genus.

Results

Nematode genera, abundance and frequency of occurrence

Seventeen plant parasitic nematode genera were found in the soils from sugarcane fields at Section 10 (Table 3). Sixteen genera were identified in soils collected from root zones of stunted cane and soils collected in inter-rows from healthy growing cane areas, while 13 nematode genera were identified in soils collected from the root zones of healthy cane and soils from inter-rows in stunted cane areas (Table 3). *Rotylenchus* sp. was found in soils around stunted cane roots only, while *Ditylenchus* sp. and juveniles of *Meloidogyne* sp. were not present in the soils around the root zones of healthy sugarcane plants. *Longidorus* sp. was present in soils collected in the inter-row of both stunted and healthy growing cane. Unidentified non-parasitic nematodes were also present in soils collected from the inter-row in areas with stunted cane and healthy growing cane, while none were identified in soils from the sugarcane root zones.

Table 3. Relative abundance/richness and diversity of nematodes in soils collected from sugarcane root zones and cane inter-rows.

Genus	Soils around or from				
	Healthy cane root zone ($-p_i \ln p_i$)	**Stunted cane root zone ($-p_i \ln p_i$)**	**0.75 m from healthy cane line ($-p_i \ln p_i$)**	**0.75 m from stunted cane line ($-p_i \ln p_i$)**	**$\sum p_i(\ln p_i)$**
Scutellonema	0.37	0.37	0.32	0.34	1.39
Helicotylenchus	0.28	0.16	0.10	0.15	0.69
Rotylenchulus	0.20	0.30	0.29	0.28	1.06
Pratylenchus	0.17	0.19	0.25	0.20	0.81
Criconemella	0.19	0.13	0.35	0.33	1.00
Trichodorus	0.08	0.05	0.08	0.11	0.32
Meloidogyne	0.00	0.03	0.01	0.00	0.04
Xiphinema	0.07	0.10	0.06	0.04	0.27
Tylenchorhynchus	0.31	0.30	0.10	0.16	0.87
Aphelenchus	0.11	0.09	0.11	0.13	0.44
Ditylenchus	0.00	0.01	0.002	0.003	0.01
Hemicycliophora	0.01	0.07	0.005	0.00	0.08
Aphelenchoides	0.05	0.05	0.01	0.01	0.11
Tylenchus	0.02	0.03	0.03	0.01	0.09
Hoplolaimus	0.03	0.06	0.002	0.00	0.09
Longidorus	0.00	0.00	0.001	0.01	0.011
Rotylenchus	0.00	0.05	0.00	0.00	0.05
Non-parasitic	0.00	0.00	0.28	0.28	0.56
H' = $\sum p_i(\ln p_i)$	1.88	1.97	1.99	2.05	
Number of genera	13	16	16	13	

Three nematode genera were identified in sugarcane roots (Figure 3). *Meloidogyne* sp. was found in roots collected from stunted sugarcane plants only. *Pratylenchus* sp. and *Rotylenchulus* sp. were found in roots from both healthy and stunted sugarcane plants (Figure 3), with significantly more in the roots of stunted sugarcane.

Pratylenchus sp. was identified in all samples of sugarcane roots (healthy and stunted) (Figure 4), while *Meloidogyne* sp. was identified in ~10% of root samples collected from stunted cane

plants only. *Rotylenchulus* sp. was identified <20% of root samples collected from both healthy and stunted sugarcane plants (Figure 4).

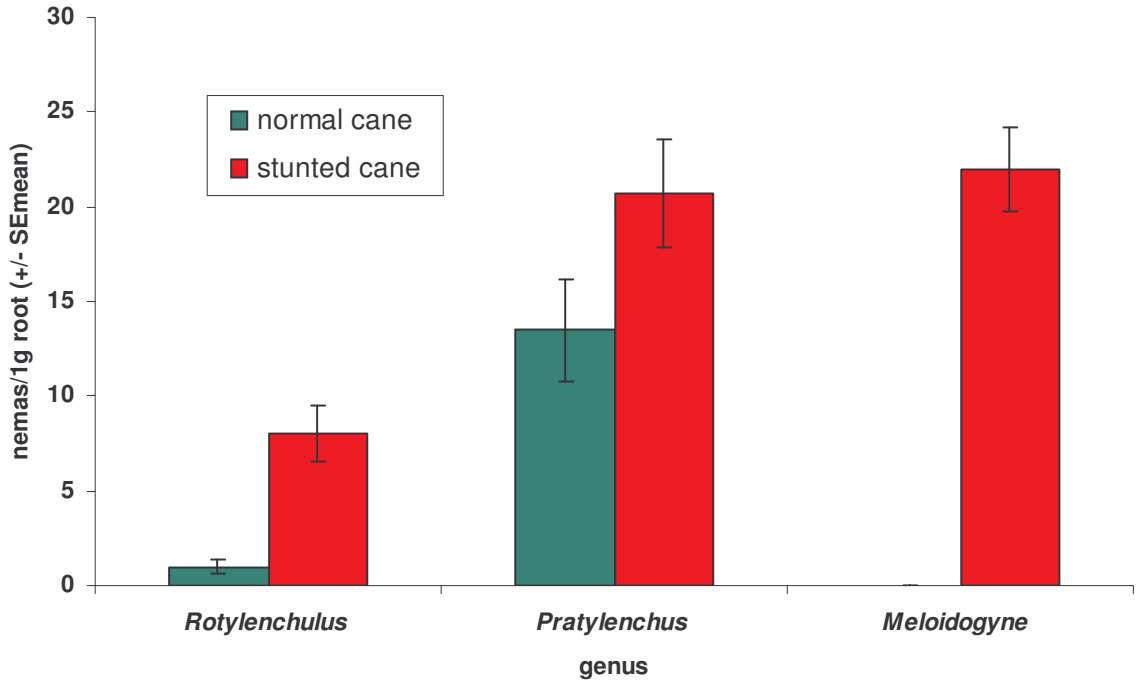

Figure 3. Average number of nematodes recovered from roots of healthy and stunted sugarcane.

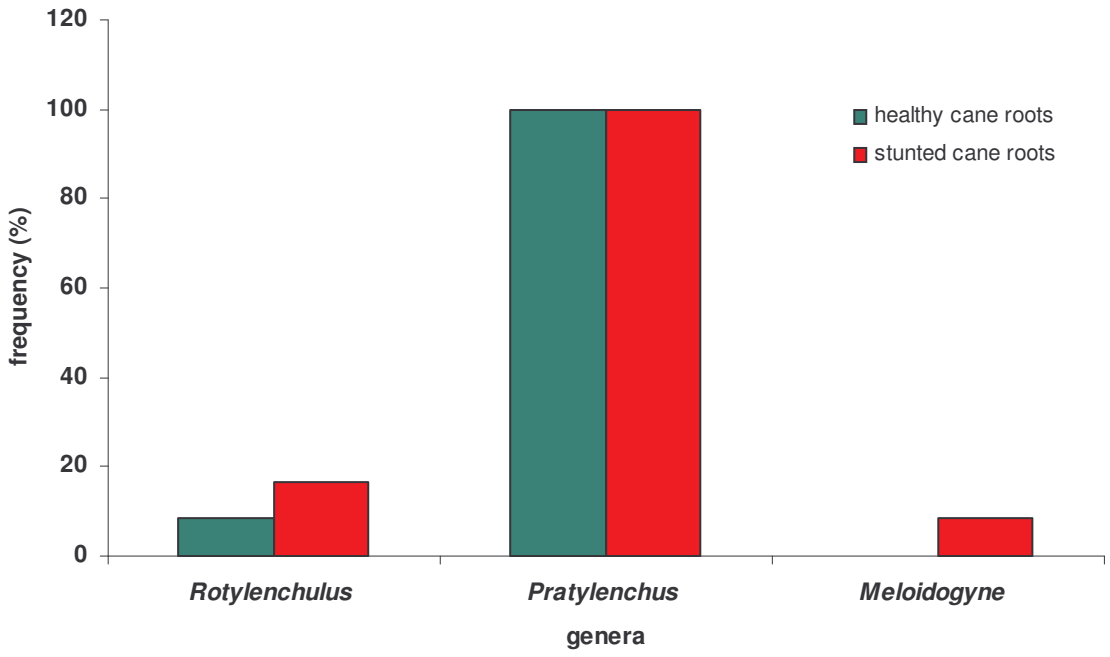

Figure 4. Percentage frequency of occurrence of nematode genera recovered from roots of healthy growing and stunted sugarcane.

Generally more plant nematodes were identified in soils collected from sugarcane roots zones compared to soils from the sugarcane inter-rows (Figure 5). Significantly more nematodes

were identified in soils collected from root zones of stunted cane compared to soils from healthy cane root zones (Figure 5) (t test, p=0.002). *Scutellonema* sp. and *Rotylenchulus* sp. were the most abundant nematodes (>120 nems/100 g soil) in soils collected from root zones of stunted sugarcane (Figure 5). Significant numbers of *Pratylenchus* sp., *Meloidogyne* sp., *Tylenchorhynchus* sp., *Hemicycliophora* sp., *Aphelenchoides* sp., *Hoplolaimus* sp. and *Rotylenchus* sp. were also identified in soils collected from root zones of stunted sugarcane plants. There were no significant differences in nematodes numbers recovered from soils collected from the inter-rows from healthy growing areas compared to stunted cane areas (Figure 5) (t test, p=0.15).

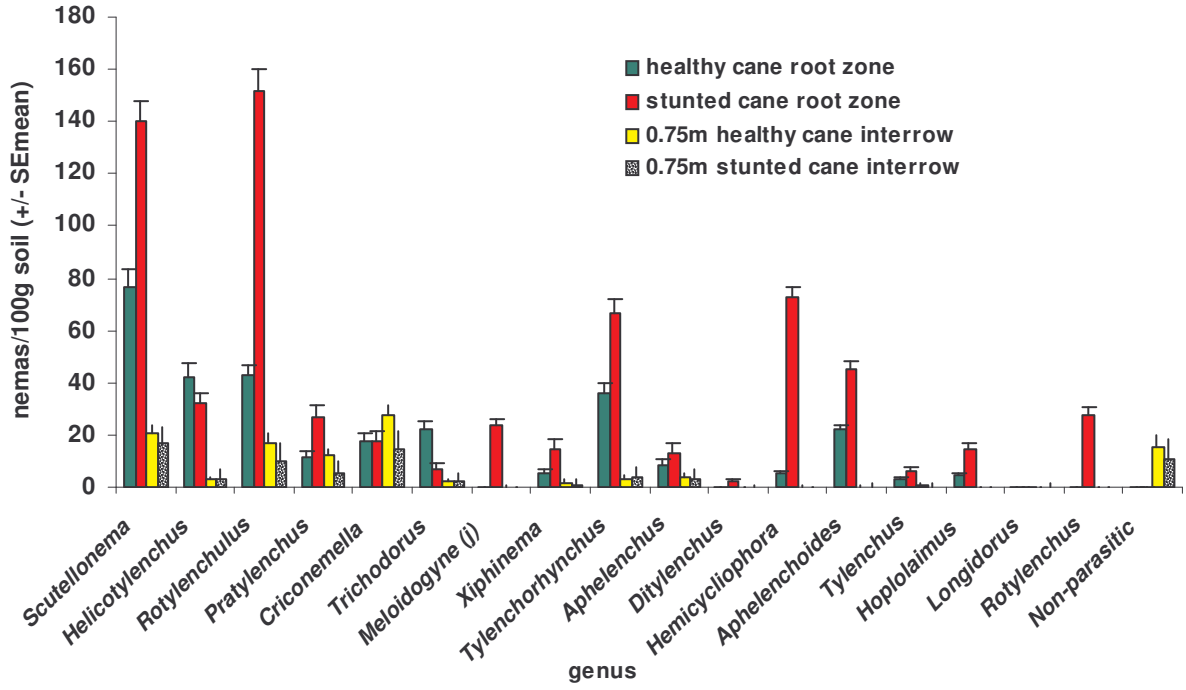

Figure 5. Average number of nematodes recovered from soils collected from cane root zones and 0.75 metres from cane rows in areas with healthy and stunted sugarcane.

Scutellonema sp. was identified in >90% of soil samples collected from all four habitats (root zones of healthy and stunted cane, cane inter-rows in healthy and stunted cane areas) (Figure 6), while *Pratylenchus* sp. and *Tylenchorhynchus* sp. were identified in >90% of soil samples from stunted and healthy cane root zones only (Figure 6). *Criconemella* sp. was identified in >90% of soil samples collected from stunted cane root zones and samples from the inter-rows (Figure 6). *Helicotylenchus* sp., *Trichodorus* sp., *Xiphinema* sp. and *Aphelenchus* sp. were identified in >60% of soils samples collected from stunted cane root zones.

Meloidogyne sp. juveniles, *Ditylenchus* sp., *Hemicycliophora* sp., *Aphelenchoides* sp., *Longidorus* sp., *Rotylenchus* sp. and non-parasitic nematodes were identified in <20% of the soil samples collected from all four habitats (Figure 6).

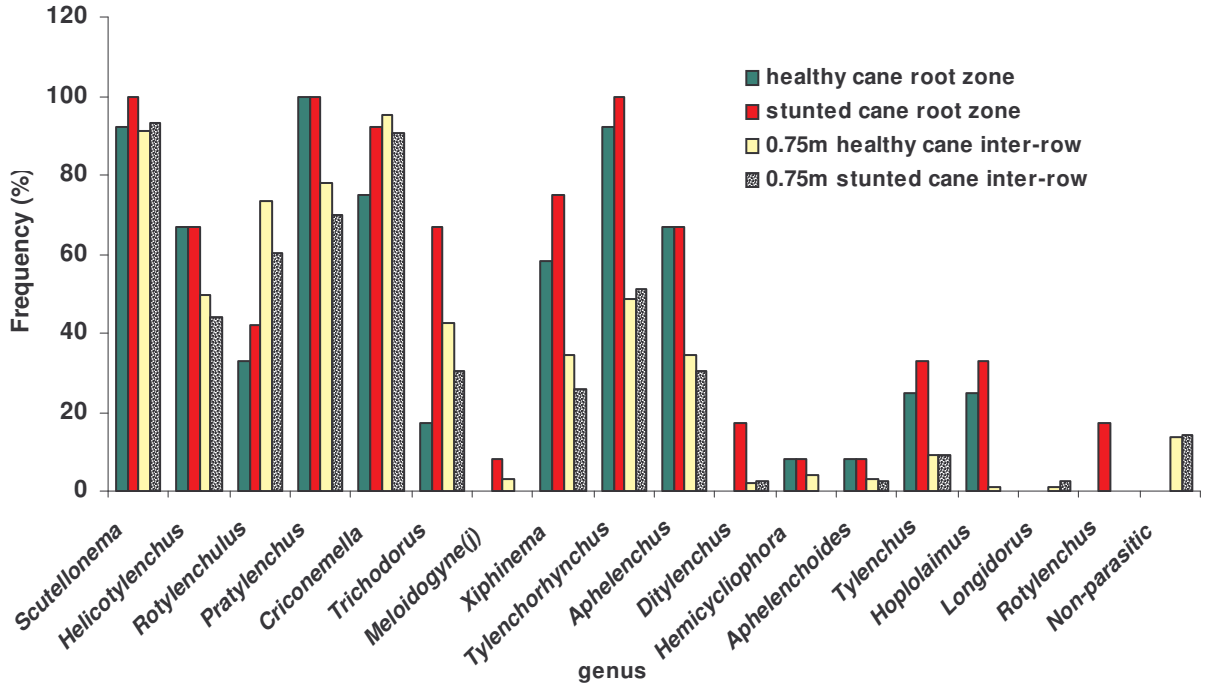

Figure 6. Percentage frequency of occurrence of nematodes identified in soils from inter-rows and root zones of healthy and stunted sugarcane.

Nematode diversity and evenness

Scutellonema sp. and *Rotylenchulus* sp. were present in soils collected from all four habitats in almost equal proportions (Table 3). *Pratylenchus* sp. and *Criconemella* sp. were present in higher proportions in soils collected from the inter-rows in both healthy growing and stunted cane areas, while *Tylenchorhynchus* sp. was present in higher proportions in soils collected from root zones of both healthy and stunted cane. *Helicotylenchus* sp. was present in higher proportions in soils collected from root zones of healthy cane. *Trichodorus* sp. though present in low proportions, was evenly distributed in all four habitats (Table 3). The index of diversity in all four habitats was ~2, indicating no significant differences in the nematode richness among the soils collected from the four habitats (Table 3).

Discussion

Plant parasitic nematodes have been identified in sugarcane (Prasad, 1972) and these include nematodes from Tylenchoidea, Aphelenchoidea and Dorylaimida groups. In this current study, there were twelve genera from the Tylenchoidea group, two Aphelenchoids and three of the Dorylaimida. Similarly, Martin (1962) identified eighteen genera of nematodes from roots and soils around root zones collected from sugar estates at Triangle, Chirundu, Hippo Valley, in the then Southern Rhodesia (Zimbabwe), though Martin's study covered a wider area.

This study has identified a wide range of sugarcane nematodes some of which could be parasitic or are potentially parasitic to sugarcane. Hippo Valley Sugar Estates has been in

existence for over 40 years, and section 10 was mostly a citrus grove, before the introduction of sugarcane in fields 1051 and 1053 in 1998. Soil samples from around citrus trees throughout the world have revealed the presence of numerous species of nematodes (Cohn, 1972). Some nematode genera associated with citrus elsewhere were identified in this study. These are *Trichodorus, Meloidogyne, Rotylenchus, Hemicycliophora*, *Criconemella,* and *Helicotylenchus*. Nematodes such as *Pratylenchus, Xiphinema* and *Aphelenchoides* also infest citrus and were also identified in this study. It can be presumed that the enhanced nematode community in these sugarcane fields could be due to the citrus that was grown previously. Also, the most significant parasitic nematodes of sugarcane viz. *Meloidogyne* sp., *Xiphinema* sp., *Paratrichodorus* sp. and *Pratylenchus* sp. were identified in this study.

Most of the nematodes collected in this study were ectoparasites. Generally ectoparasitic nematodes feed superficially on plant roots, and their feeding could cause economic loss (Anon, 2000) by inhibiting root growth and disrupting water and nutrient uptake. This would result in poor crop performance and crop conditions such as lack of vigour, stunting and reduced yields (Anon, 2000). Significant numbers of *Scutellonema* sp., *Rotylenchulus* sp., *Tylenchorhynchus* sp. and *Hemicycliophora* sp. were identified in soils around stunted cane root zones. *Pratylenchus* sp., juveniles of *Meloidogyne* sp., *Aphelenchoides* sp., *Rotylenchus* sp., *Xiphinema* sp. and *Hoplolaimus* sp. were also associated more with stunted cane root zones than healthy cane. This could be the possible cause of stuntedness observed in the sugarcane fields.

Meloidogyne sp., a sedentary endoparasite, *Rotylenchulus* sp., a sedentary semi-endoparasite and *Pratylenchus* sp. were found in greater numbers in roots of stunted cane. General nematode damage on plant roots affects the efficient uptake of water and nutrients, hence there is loss of plant vigour. This could have also contributed to the stuntedness of the sugarcane.

Associations have been reported to occur between nematode species and species of fungi, bacteria or viruses (Prasad, 1972). *Xiphinema, Longidorus* and *Trichodorus* are known viral vectors in other crops, while close associations have been reported for *Meloidogyne, Helicotylenchus* and *Pythium arrhenomanes* in sugarcane (Prasad, 1972). Similarly *Pratylenchus zeae* and *Phytophthora megasperma* have been found to affect sugarcane growth (Prasad, 1972). It is possible that the stuntedness of sugarcane observed in these fields could be due nematodes-diseases complexes.

Higher numbers of *Helicotylenchus* sp. were identified in soils from healthy cane root zones. Cadet *et al*. (2002a, b) reported a similar observation in sugarcane. Spaull and Cadet (2003) reported that at low numbers most plant feeding nematodes had little deleterious effect on plants; they may even have a stimulatory effect on root growth. It has been reported that under a set of soil, temperature and moisture conditions any crop can support a specific number of individuals of a given nematode species without being damaged (Spaull *et al.,* 2003), but will suffer damage when the population density exceeds this tolerance limit. Williams (1969) gave a range of tolerance limits for various species of nematodes in sugarcane (e.g. 5000 per 100 g soil for *Tylenchorhynchus martini* on 8-month old sugarcane and 255 g of root tissue for *Pratylenchus zeae*).

In this study, nematodes were identified in soils collected in the inter-rows in both stunted cane areas and healthy cane areas. There were significant differences in the numbers collected from the different habitats. Nematodes could be maintained in these inter-row areas by the

presence of susceptible weeds. It has been reported that the reniform nematode *Rotylenchulus parva* infests both sugarcane and the common weed *Bidens pilosa* (blackjack) in Mauritius (Williams, 1969). Berry and Wiseman (2003) and Berry *et al*. (2005) also highlighted the significance of the alternative host plants and weeds in nematode population fluctuations. Heyns (1971) noted that most of the plant parasitic and potentially parasitic nematodes in South Africa were present in uncultivated veld and could be ubiquitous in sandy soils.

Indices of diversity were determined for the four habitats that were sampled in this study. There were no differences in nematode diversity among the habitats. However, there were differences in the evenness of distribution of the various nematode genera in the fields. *Scutellonema* sp. was the most abundant nematode and was evenly distributed in all habitats. Similarly, for *Rotylenchulus* sp. Proportionally, more *Pratylenchus* sp. and *Criconemella* sp. were found in the inter-rows in both stunted and healthy cane area. The differences in abundance and diversity could be due to several factors. Sugarcane is a monoculture crop, which is ratooned over several years. The nematodes present in the soil of sugarcane have adapted to feeding and reproducing on sugarcane, since it is a host that is readily available (Cadet *et al*., 2002a). However, the plant type and length of growth of plants/weeds in the inter row may change over time and even within seasons due to weeding and/or herbicide usage in the inter row. This could have an impact on the type and number of nematodes present in this area.

Inference

Plant parasitic nematodes were identified in soils collected from sugarcane fields and from sugarcane roots. Significantly more nematodes were identified in soils collected from stunted cane root zones. The presence of these nematodes is a likely factor resulting in yield loss to this sugarcane. Likely ways of remedying this situation could be the use of nematicides in the succeeding ratoon crops and/or replanting with more tolerant sugarcane varieties. This requires further investigation.

Acknowledgements

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