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NEMATOLOGICAL ENVIRONMENT AND SELECTION PRESSURE ON SIX SASRI RESEARCH FARMS

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Abstract

Current estimates indicate that the loss in yield of sugarcane from root-feeding nematodes amounts to approximately R250 million per year. Tolerant varieties provide an inexpensive and long-lasting option to reduce crop loss from nematodes. Currently, genotypes are selected on the SASRI research farms without direct attention being given to their tolerance or susceptibility to nematodes. Not much is known about the nematode communities that exist on the research farms and whether these nematode communities exert sufficient selection pressure on the genotypes in the selection programme.

The objectives of this work were (i) to characterise the plant breeding selection sites according to their nematode communities, and (ii) to measure the effect that nematodes have on the yield of varieties at plant breeding selection sites and thus identify the selection pressure for nematode tolerance.

To achieve this, the 2nd stage single lines at six research farms were surveyed for nematode populations. Results showed that Empangeni, Gingindlovu and Kearsney had similar nematode profiles, whereas other research farms exhibited differences in nematode communities. For example, Bruyns Hill and Glenside had higher numbers of *Helicotylenchus,* whereas Pongola had higher numbers of *Hemicycliophora* and *Rotylenchulus*. The common variety at all sites, NCo376, exhibited a similar nematode community to other sugarcane clones at a particular site, but a substantially different community between sites. Yield data from single line trials showed that nematode communities influenced selection at four of the six sites. Two variety x nematicide trials were planted to assess the loss due to nematodes. At Empangeni, the yield responses (which reflect crop loss) in the plant crop ranged from 10 to 27%. At Gingindlovu, the responses in the plant crop ranged from 44 to 52%.

Keywords: nematodes, plant breeding, selection, sugarcane, yield

Introduction

Plant parasitic nematodes are a problem throughout the South African sugar industry, not only on the weaker sandy soils but also on the better soils. Losses due to nematodes are estimated to be more than 1.6 million tons of cane (equivalent to R250 million) per year (Spaull and Cadet, 2003). The response to applying a nematicide on the poor sandy soils justifies the cost of treatment, but on the better soils responses are smaller and treatment is not always cost effective. The effect of nematodes is not confined to the annual loss in yield, but also has a major impact on sustained production by limiting the number of high yielding ratoon crops.

This, in turn, has a disproportionate effect on income per hectare and per ton (personal communication[1]).

Aldicarb, the active ingredient in the nematicide Temik, is the most effective and widely used nematicide in the sugar industry. It is highly poisonous, which, together with its misuse, has brought into question its continued availability, and alternative methods of control are required. Addressing the 'nematode problem' from a plant breeding perspective has not received the same attention given to other pests and diseases (*viz*. the stalk borer *Eldana saccharina* Walker (Lepidoptera: Pyralidae), and smut and mosaic diseases). Certainly, breeding for resistance is used in many other crops to overcome the problem from nematodes (Star *et al*., 2002). However, sugarcane is attacked not only by one, but by several plant parasitic species, each of which interact with the host plant in different ways (Cadet and Spaull, 2005). Breeding for combined resistance, or even resistance to the more important components of such a community, is therefore likely to be difficult. Breeding and selecting varieties for tolerance to nematodes would be a better option. This is particularly so in view of the observed considerable benefit derived from growing tolerant rather than susceptible varieties in situations where nematodes are a problem (Spaull and Cadet, 2003).

At present there are a few commercial varieties that grow better than others in situations where nematodes are a problem. These include N12, N23 and N25 (Spaull and Cadet, 2003). The initial selection of these varieties was made on the basis of their performance in different environments and according to their reaction to diseases and pests (excluding nematodes). It is likely that, in the process of selecting the best varieties, some random selection of nematode tolerance also occurs. Data from field trials show that, compared with many other varieties, N12 favours the spiral nematode *Helicotylenchus dihystera*, and yields are greater where this nematode occurs in large numbers. Perhaps more than coincidentally, N12 was selected at Windy Hill in the Midlands, which is an area where this nematode (*H. dihystera*) predominates. Varieties N23 and N25 were selected on the better soils at the Pongola research station. Although numerous nematode species occur in such soils, it seems likely that tolerance to nematodes would be considerably enhanced if genotypes were specifically bred for this attribute and selected in soils with a greater nematode pressure. It might be argued that such pressure already occurs on the research farms that are situated on lighter textured soils, *viz*. Gingindlovu, Glenside and Kearsney. However, there is no information on the nematode communities that occur, nor the constraints that they impose on crop growth, at any of the plant breeding selection sites on the research farms.

To address this situation, a project was initiated in 2005 to characterise the plant breeding selection sites at the six South African Sugarcane Research Institute (SASRI) research farms (i) to survey the nematodes in the first ratoon crop of Stage 2 (single lines) of the plant breeding programme at each of the research farms, and (ii) to measure the existing selection pressure at two of the research farms as determined by the response of commercial varieties to the removal of practically all the nematodes.

Materials and Methods

Characterising the nematode communities at plant breeding selection sites

From mid-July to early October 2005, soil samples were collected from Stage 2 plant breeding trials on and around the SASRI research farms at Bruyns Hill, Empangeni, Gingindlovu, Glenside, Kearsney and Pongola (Table 1). The samples were taken from the

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first ratoon crop of between 34 and 126 genotypes, and from the standard variety NCo376 (Table 1). A total of 513 soil samples were collected for nematode assay. Based on maps of the trial sites, the single lines of the clones from which samples were taken were pre-selected such that they were spread over all, or a large part, of the trial area in a grid-like fashion. The exception was the BSL site at Bruyns Hill, where only one third of the trial area was included in the survey. The lines were selected without knowing the history of the clones. At intervals during the sampling, the pre-selected clone was substituted by an adjacent line of the standard variety, NCo376, such that between 9 and 20% of the sampled lines were of this variety. The soil samples were collected shortly before harvest of the 1st ratoon of all the single line sites, except at Pongola (FSL03) where the cane was about one month into the 2nd ratoon.

Nematode samples were collected adjacent to the sugarcane stalks, at depths of 5-15 cm below the soil surface. The nematodes were extracted from 200 ml of soil using the elutriation technique (Seinhorst, 1962). The nematode genera were enumerated under the microscope.

Subsamples of the soil collected from the Stage 2 trials were used to measure the chemical and physical components of the soil at each site. The soil and nematode data, and the yield data obtained by the Plant Breeders, were subjected to regression analysis and principal component analysis to identify associations between nematodes, the soil environment and the yield of the genotypes.

Table 1. Research farms, trial codes and number of samples taken from Stage 2 single lines.

Site	Stage 2 (single lines)	No. of samples
Midlands – Bruyns Hill	BSL02	34
Empangeni	TSL03	126
Gingindlovu	USL03	82
Midlands – Glenside	SSL02	91
Kearsney	KSL03	100
Pongola	FSL03	80

Measuring the selection pressure at plant breeding selection sites

Two similar field trials were planted, one at the Empangeni research farm and the other at the Gingindlovu research farm, to determine the response of nematode-susceptible and nematode-tolerant varieties to the almost complete elimination of nematodes. The site at Empangeni had been under a weed fallow for more than 12 months, whereas the site at Gingindlovu had been without sugarcane for only a few weeks. The soil at the Empangeni trial site was a clay with 44% clay, 18% silt and 38% sand. At Gingindlovu the soil was a clay loam with 28% clay, 25% silt and 47% sand. At both sites N19 and N27, rated as susceptible to nematodes, and NCo376, considered tolerant, were planted with and without nematicide (Temik 15G) in a split plot design. Plots comprised 5 rows x 6.5 m (Empangeni) and 5 rows x 7.5 m (Gingindlovu) and the treatments were randomised in blocks. The nematicide was applied at 28 kg/ha in the furrow at planting and at 58 kg/ha at 2, 4 and 10 weeks later. Soil and root samples were collected from all plots for nematode analysis at both sites at 10, 24 and 47 weeks after planting. After 12 months, the plant and ratoon crops were harvested. The cane was burnt at Empangeni and cut green at Gingindlovu. The centre three rows were weighed, and a 12 stalk sub-sample collected per plot to measure sucrose, fibre and pol content. In the first ratoon crop, nematicide was applied once, at 20 kg/ha, one month after harvest and nematode samples were collected at 12, 15, 27 and 44 weeks after harvest. After just over 11 months growth, both trials were harvested. The harvest procedure was the same as that of the plant crop.

Results

Soil physical and chemical characteristics

The clay content of the soils at the single line sites ranged from 9% (Glenside) up to 37% (Empangeni) (Figure 1). The soil at Glenside was classified as a sandy soil, whereas the other five sites were loam to clayey soils. All of the soils were acidic, with the pH ranging from 3.98 (Gingindlovu) to 6.03 (Pongola). There was little difference in levels of P, Na, Zn and Al between the sites. Pongola had the highest levels of K, Ca and Mg, and the lowest levels of Fe (Table 2).

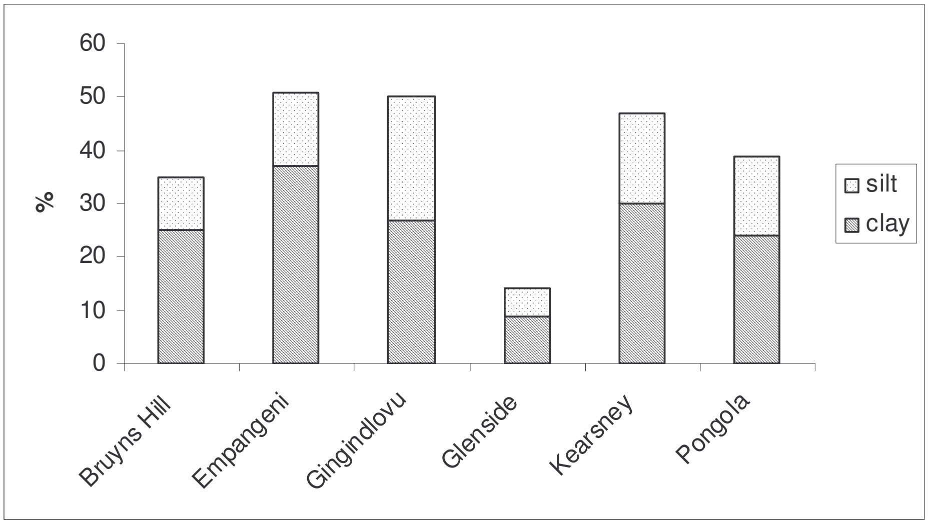


Figure 1. Soil physical characteristics of plant breeding sites.

Table 2. Soil chemical characteristics of plant breeding sites.

Site	pH	P (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	Na (ppm)	Zn (ppm)	Fe (ppm)	Al (ppm)
Glenside	5.22	41	97	158	35	17	1	207	13
Bruyns Hill	5.35	39	245	499	125	20	3	130	20
Gingindlovu	3.98	29	153	313	99	29	4	179	43
Kearsney	4.28	22	118	594	230	39	3	168	20
Empangeni	4.08	43	144	510	173	21	4	59	34
Pongola	6.03	54	271	889	417	33	4	4	6

Nematode communities at different sites

Nematode genera were examined based on their frequency (% of samples containing these genera) and their abundance (average number per positive sample). Nematode genera that occurred in more than 30% of the samples and in abundance of more than 300/litre of soil were considered to be 'well adapted to the plant and in need of control measures' (Figure 2).

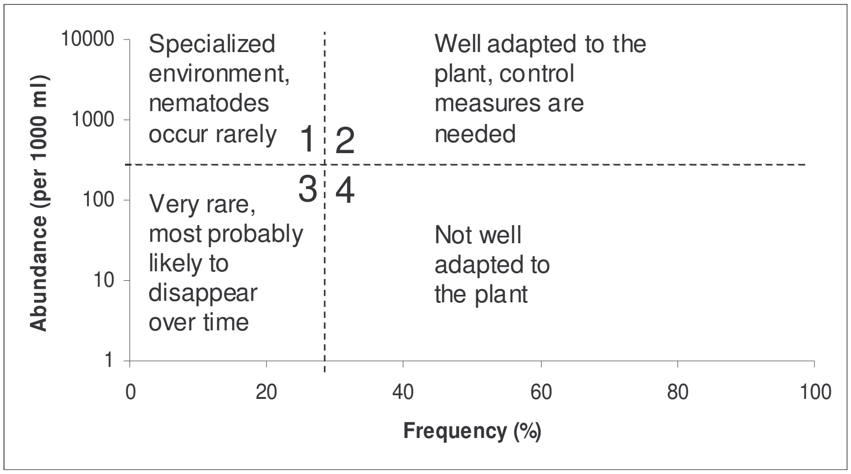


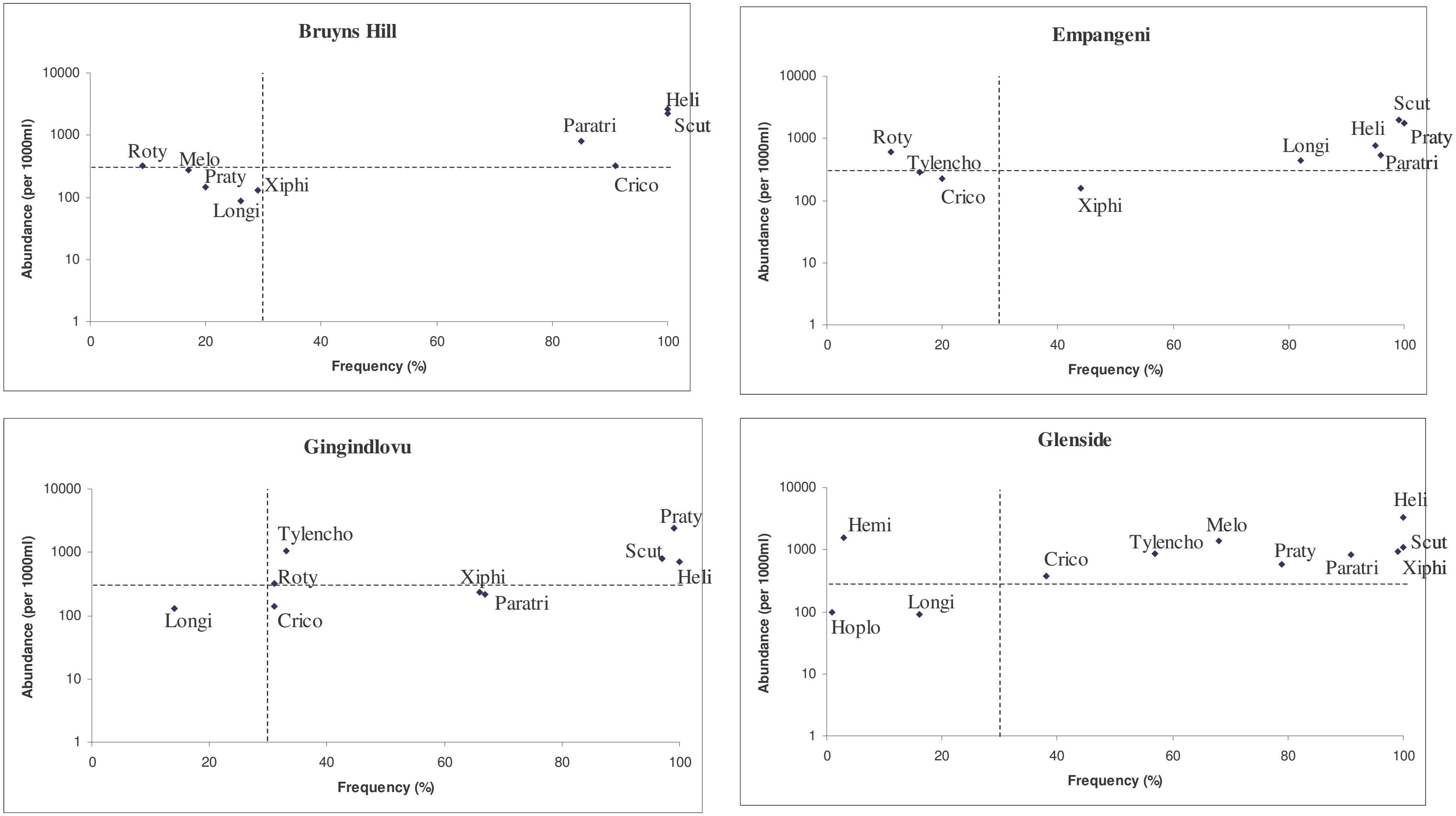
Figure 2. Explanation of the chart used by Fortuner and Merny (1973) to study the frequency and abundance of nematode genera from survey data (compare this to sugarcane data shown in Figure 3).

Results showed differences in the numbers of frequent and abundant genera between the research farms (Figure 3). For example, Bruyns Hill had four genera that could be considered as 'frequent and abundant', *viz. Criconemoides*, *Helicotylenchus*, *Paratrichodorus* and *Scutellonema*. Empangeni had five such genera, Gingindlovu five, Glenside eight, Kearsney six, and Pongola four. All six sites had *Scutellonema* in more than 90% of the samples. *Helicotylenchus* and *Pratylenchus* were the next most common genera. *Xiphinema* was common at Glenside, and *Rotylenchulus* at Pongola (Figure 3).

When comparing the nematode communities between research farms (Figure 4A, B), there was little difference between Bruyns Hill, Empangeni, Gingindlovu and Kearsney. These four farms had their gravity centre clustered around the middle of the factorial plan (Figure 4B). Pongola and Glenside, however, had different nematode communities, with Pongola having a community dominated by *Rotylenchulus* and Glenside a community dominated by *Xiphinema* (Figure 4A, B). Furthermore, when the data for all sugarcane genotypes were split and compared to the standard (NCo376), it was apparent that the nematode communities were more strongly influenced by site than by genotype (Figure 4C). NCo376, a variety common to all six sites, had distinct nematode communities that differed between sites. Thus the nematode community of NCo376 was more similar to the communities associated with the clones at a particular site than to the community associated with NCo376 at other sites.

Nematode communities and the yields of single lines

Regression analysis of nematode communities and yield of genotypes at single line sites showed significant negative correlations between certain nematode genera and cane yield (Table 3). Plant-feeding nematodes were found to influence selection based on cane yield at four of the six sites (Table 3). Furthermore, at only one site (Pongola) did nematodes exert a significant negative effect on cane quality (% ERC) (data not shown).



Crico=*Criconemoides*, Heli=*Helicotylenchus*, Hemi=*Hemicycliophora*, Longi=*Longidorus*, Melo=*Meloidogyne*, Paratri=*Paratrichodorus*, Praty=*Pratylenchus*, Roty= *Rotylenchulus*, Scut=*Scutellonema*, Tylencho=*Tylenchorhynchus*, Xiphi=*Xiphinema*

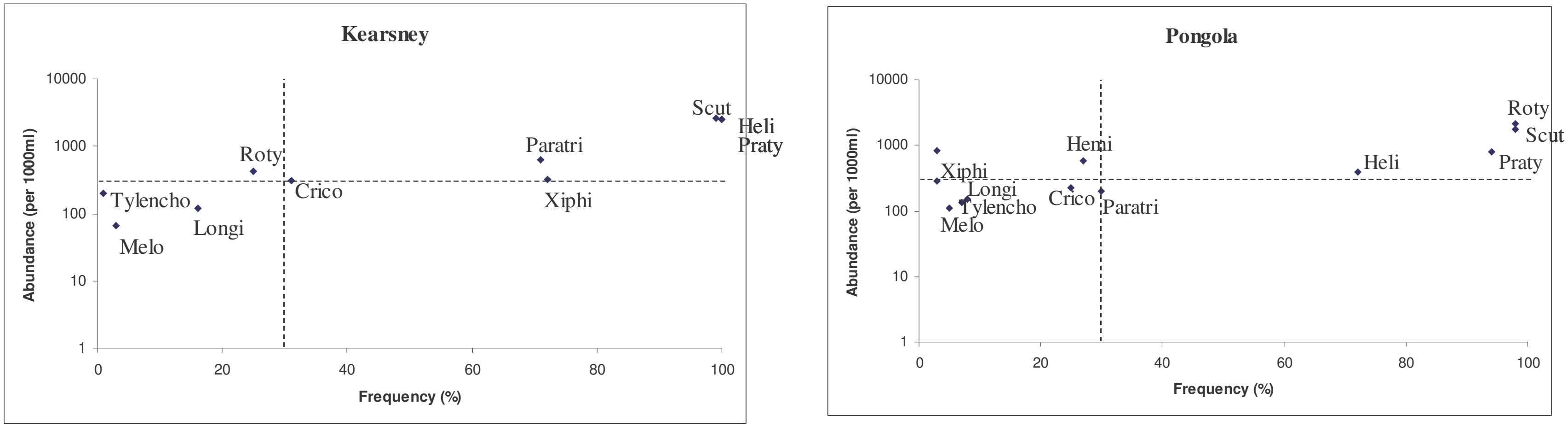


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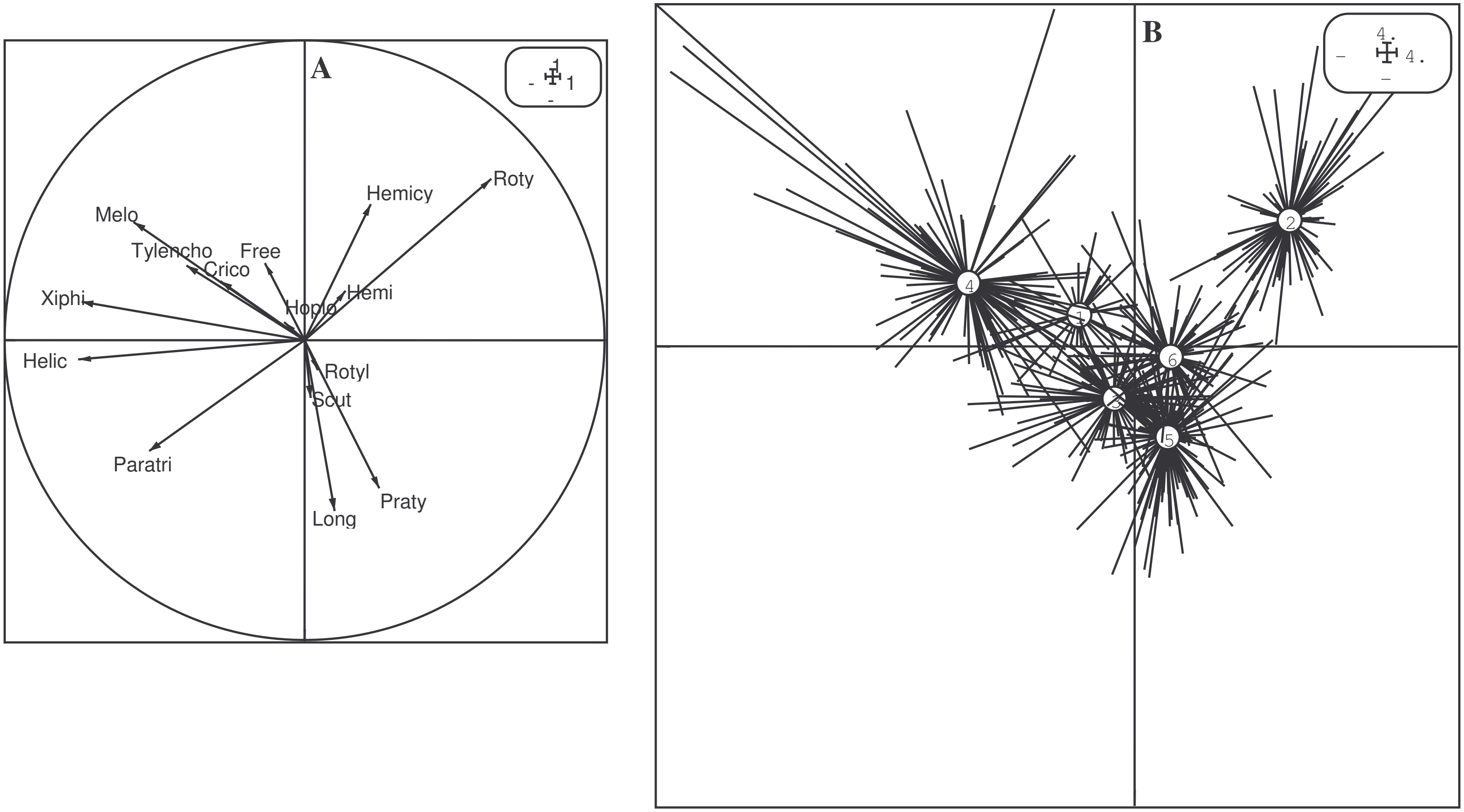
Figure 3. Classification of nematode populations associated with the single lines on the research farms, according to their frequency (% of samples containing these nematodes) and abundance (average number per positive sample).



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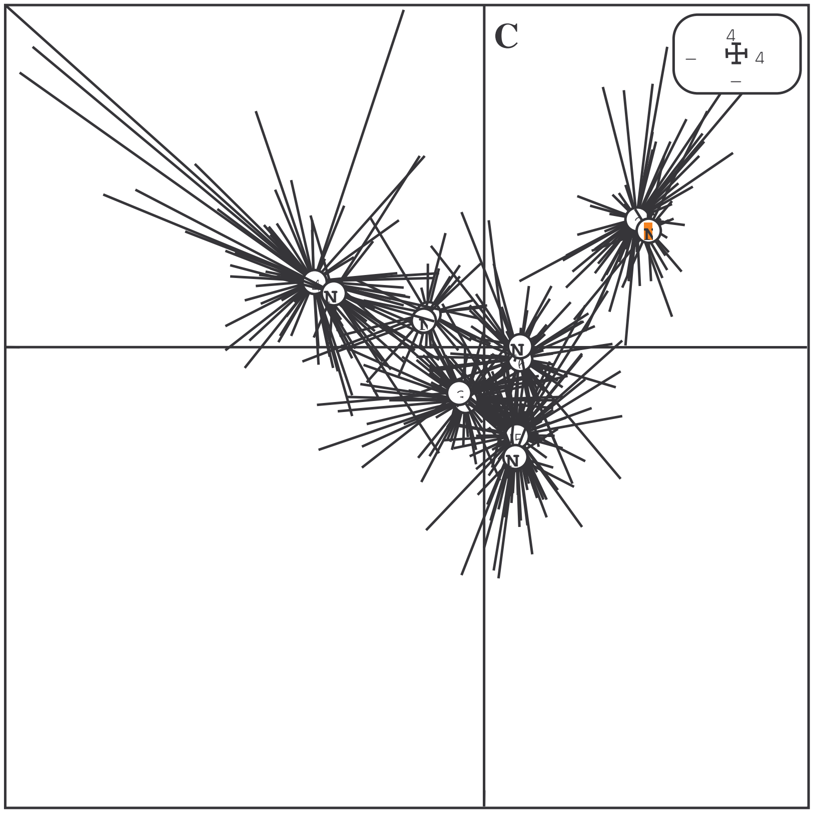

Figure 4 (A) Correlation circle showing the relationship between the genera comprising the nematode communities at the six sites; (B) Factorial map showing the relationships of the six different sites in terms of their nematode communities; (C) Factorial map showing the relationship of NCo376 (N) to other genotypes at each of the sites. Site codes: 1=Bruyns Hill, 2=Pongola, 3=Kearsney, 4=Glenside, 5=Empangeni, 6=Gingindlovu.

Table 3. Regression analysis showing significant negative correlations between certain nematode genera and yield in tons cane/ha.

Site	P-value	Nematode genus
Bruyns Hill	0.56	–
Empangeni	0.05	*Helicotylenchus, Scutellonema*
Gingindlovu	0.05	*Xiphinema*
Glenside	0.04	*Xiphinema*
Kearsney	<0.001	*Pratylenchus, Criconemoides*
Pongola	0.1	–

Nematode selection pressure

Application of Temik to both the plant and ratoon crops had a substantial effect in reducing nematode numbers in the treated plots (Figure 5). Although never reduced to zero, nematode numbers were relatively low between 10 and 24 weeks after planting, for both trials, and for most of the crop cycle for the ratoon crop at Empangeni (Figure 5). In the plant crop, average untreated yields were greater at Empangeni (112 t cane/ha) than at Gingindlovu (83 t cane/ha) (Table 4). At both sites, one or more of the three cane varieties responded to treatment with the nematicide ($P<0.05$) in the plant crop. The response was significant for all three varieties at Gingindlovu, but only for N19 at Empangeni. The average tons cane/ha response to treatment in the plant crop was greater at Gingindlovu (48%) than at Empangeni (19%). The average tons ERC/ha response to treatment reflected that for tons cane, although the difference was greater; 64% response at Gingindlovu and 16% at Empangeni. In the ratoon crops, the yields were lower, the magnitude of the differences was smaller and none of the varieties showed a significant response to nematicide treatment (Table 4). Also, whereas there was a tendency for yields to be greater and response to treatment least in NCo376 in the plant crops, this was not the case with the ratoons.

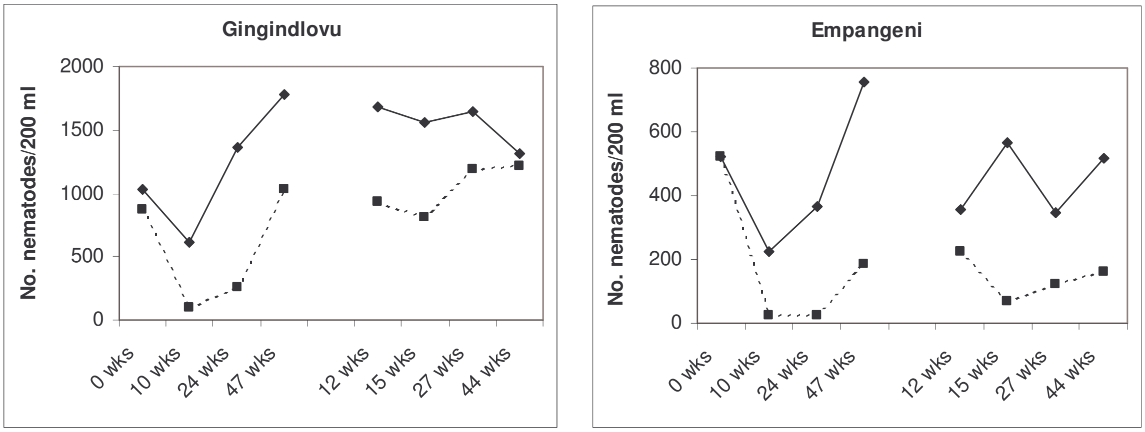

Figure 5. Nematode numbers for the control (solid lines) and nematicide-treated (dashed lines) sugarcane plots at various sampling times for the plant and ratoon crops of the tolerance trials at Ginginglovu and Empangeni.

Table 4. Response to nematicide treatment for plant and ratoon crops in trials at Gingindlovu and Empangeni. Numbers followed by an asterisk (*) are significantly different to the control (P<0.05).

		Variety and treatment	**Cane (t/ha)**	**Response (%)**	**ERC (t/ha)**	**Response (%)**
GINGINDLOVU	Plant crop	376 Control	90		8.9	
		376 Treated	130*	44	14.4*	62
		N19 Control	83		9.3	
		N19 Treated	126*	52	15.1*	62
		N27 Control	76		7.6	
		N27 Treated	112*	49	12.8*	68
	Ratoon	376 Control	57		5.9	
		376 Treated	59	4	5.2	-12
		N19 Control	50		5.1	
		N19 Treated	57	14	5.4	6
		N27 Control	58		6.3	
		N27 Treated	68	17	7.1	13
EMPANGENI	Plant crop	376 Control	140		19.1	
		376 Treated	154	10	9.7	3
		N19 Control	110		15.5	
		N19 Treated	139*	26	19.5*	26
		N27 Control	86		11.1	
		N27 Treated	105	22	13.7	23
	Ratoon	376 Control	87		8.5	
		376 Treated	90	3	8.4	-1
		N19 Control	95		10.1	
		N19 Treated	93	-2	9.4	-7
		N27 Control	78		8.6	
		N27 Treated	98	26	10.7	24

376 = NCo376, ERC = estimated recoverable crystal

Discussion

It is apparent from the results that only one of the research farms (Glenside) is situated on what can be termed a ‘sandy’ soil. This is typically a soil with <10% clay where nematode damage has been shown to be the most economically controllable and for which most research data exists. At Glenside, the nematode community was similar to that which occurs on the sandy soils within the sugar industry, i.e. comprising species of *Criconemoides*, *Helicotylenchus*, *Meloidogyne*, *Paratrichodorus*, *Pratylenchus* and *Xiphinema* (Berry, 2007). Also at this site, there was a significant negative correlation between numbers of *Xiphinema* and sugarcane yield. This suggests that genotypes that are being selected at this farm are being exposed to considerable nematode pressure, which will most likely influence the performance of susceptible genotypes. Kearsney may be another farm where nematode selection pressure exists and is affecting selection. Here the nematode community consists of *Helicotylenchus*, *Paratrichodorus*, *Pratylenchus*, *Scutellonema* and *Xiphinema,* and there was a significant negative correlation between *Criconemoides* and *Pratylenchus* and sugarcane yield. In contrast, Pongola had a nematode community different to all the other farms. This community was not typical of a ‘sandy’ soil since it had very low numbers of *Criconemoides*, *Meloidogyne*, *Paratrichodorus* and *Xiphinema*, and high numbers of *Rotylenchulus*, a nematode not considered a major pest of sugarcane in South Africa. Thus nematode selection pressure at this farm is assumed not to be very high, which is reiterated by the lack of correlation between any nematode genera and cane yield.

Results from the single lines at Empangeni and Gingindlovu showed that at both these sites nematode numbers were negatively correlated with cane yield. Numbers of *Helicotylenchus* and *Scutellonema* were correlated with yield at Empangeni, and numbers of *Xiphinema* at Gingindlovu. However, to better assess the extent of losses, two yield response trials were planted at each site. These sites have heavier soils (27-37% clay) where yield losses due to nematodes do occur, but cannot be treated economically because of the variable, and often low, response to conventional nematicide rates. Excessively high rates (up to 10 times the standard rate) of Temik were applied to effectively control nematodes for as long as possible in the plant crops. It has been observed (Barker and Powell, 1988; Barker *et al*., 1988) that, on occasion, aldicarb can have growth promoting effects on a number of crops. However, this has not been observed in sugarcane (Spaull, 1995; Blair, 2005), and in some cases high rates can be phytotoxic to cane.

The site at Empangeni had been under a weed fallow for a year before the trial was planted, whereas Gingindlovu had had only a three-month fallow period prior to planting. This would explain the greater numbers at Gingindlovu (1 400 plant-feeding nematodes per 200 ml soil averaged over two crops) compared with Empangeni (450 plant-feeding nematodes per 200 ml soil over two crops). At both sites the very high application rates of Temik had a notable effect on numbers of nematodes, which was more apparent in some genera than others. At Empangeni there was a 20-fold decrease in the number of nematodes in the treated plots when measured at 47 weeks, three months after the last treatment, whereas at Gingindlovu, there was only a 3-fold decrease in numbers. In the ratoon crops, even though only one application of nematicide was used, there was good control of plant feeding nematodes. This control translated into significant yield responses in the plant crop. In the trial at Gingindlovu, yield responses in the plant crop ranged from 44 to 52%. At Empangeni, the responses, although evident, were not as marked and were significant only in N19. In the ratoon crops, yields from the nematicide treated plots were not higher than those of the control plots, although the nematode populations were much lower. The lack of response may be due to the poor rainfall during this season. In the plant crop the average rainfall for both trials was 1 000 mm, and in the ratoon crops rainfall decreased to 700 mm, with February, March and May experiencing almost no rainfall.

It can be inferred that nematode selection pressure exists in the plant breeding programmes on some of the SASRI research farms (Empangeni, Kearsney, Gingindlovu and Glenside), and translates into appreciable yield losses. This pressure no doubt inadvertently results in the selection of more tolerant genotypes. It is probable that genotypes on research farms such as Bruyns Hill and Pongola do not have an appreciable amount of nematode pressure. New varieties emanating from such breeding programmes will most likely be susceptible to nematodes and require nematicide treatment for economic growth on the sandier soils. However, Stage 5 variety trials are grown on a wider variety of soil types, which may preclude some of the more susceptible genotypes from being released. At present there is no available data for the Komatipoort research farm in the northern irrigated area. Additional nematode data should be collected from the other stages of the plant breeding programme. Since the Empangeni, Kearsney, Gingindlovu and Glenside research farms are relatively new (<15 years old), no new varieties from these farms have yet been released to the industry. Thus, the desired effect of releasing nematode tolerant varieties may yet occur.

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