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Distribution of pest nematodes on sugarcane in south Queensland and relationship to soil texture, cultivar, crop age and region

B. L. Blair^A, G. R. Stirling^B and P. J. L. Whittle^C

Sugar Yield Decline Joint Venture

^A Queensland Department of Primary Industries, Bundaberg Research Station, Bundaberg, Qld 4670, Australia; author for correspondence; e-mail: blairb@dpi.qld.gov.au

^B Biological Crop Protection, 3601 Moggill Road, Qld 4070, Australia.

^C Bureau of Sugar Experiment Stations, PO Box 86, Indooroopilly, Qld 4068, Australia.

Summary. Five plant–parasitic nematode species were found to be widespread on sugarcane crops surveyed in south Queensland, namely *Pratylenchus zae*, *Meloidogyne javanica*, *Paratrichodorus minor*, *Helicotylenchus dihystera* and *Tylenchorhynchus annulatus*. Apart from *Meloidogyne*, high nematode populations were found in most soil types, suggesting more extensive crop losses could be occurring than previously estimated. The most important pests were *P. zae* and *M. javanica*, as they were often found at

high densities and their pathogenicity on sugarcane is established.

Mean densities for most nematode genera did not differ significantly between sugarcane cultivars, except that fewer *Pratylenchus* were associated with cultivar CP51-21 than other cultivars surveyed. The density of *Pratylenchus* in roots was significantly higher in plant crops than in ratoon crops, whereas the density of *Paratrichodorus* was highest in first and second ratoons.

Introduction

Sugarcane is commercially produced in Queensland using an intensive system of monoculture. This system has been in use for up to 80 years in most districts, and has resulted in a decline in the productivity of sugarcane soils (Garside *et al.* 1997). Among the biological, chemical and physical factors associated with poor soil productivity, soil pathogens have been identified as important contributors to the problem (Magarey 1996). They are further implicated by the observation that soil fumigation routinely improves the root health and root volume of sugarcane grown in this system (Croft *et al.* 1984). Numerous bacteria, fungi, actinomycetes and nematodes may parasitise sugarcane roots and their relative importance probably varies both across districts and within fields (Lawrence 1984; Magarey *et al.* 1987).

Nematodes became a focus of attention in south Queensland in the late 1970s when non-volatile nematicides became available to the sugar industry. Aldicarb (Temik), ethoprophos (Mocap) and fenamiphos (Nemacur) were found to significantly increase sugarcane yields at rates which were economically

viable (Bull 1979, 1981). Sandy soils with a history of poor yields were targeted and yield improvements were most spectacular and consistent in coastal sands and sandy podzolics (<10% clay), presumably due to greater nematode damage and/or better nematicide efficacy in those soils. In the absence of subsequent research, the sugar industry concluded that nematode control was warranted only in coarse, sandy soils.

Thirty-four nematode species have been found on sugarcane in Australia (McLeod *et al.* 1994). Among them, species of root-knot (*Meloidogyne*), lesion (*Pratylenchus*), spiral (*Helicotylenchus*), stubby-root (*Paratrichodorus*), stunt (*Tylenchorhynchus*) and burrowing nematode (*Radopholus*) have been reported on damaged sugarcane in the Bundaberg district (Bull 1981). However, apart from Bull's observations at nematicide trial sites and the occasional diagnostic sample, there is little reliable information on the distribution and population densities of nematodes in the 93 000 ha of land under sugarcane in south Queensland.

We present the results of a survey which identified and quantified the pest nematodes that are present in

Table 1. Soil type categories used to describe sugarcane soils surveyed in south Queensland

Soil category number	Description ^A	Mill area	Mean particle size distribution ^B	Survey sites in the category
1	Coastal sand ridges and loamy sands	All	48:40:5:7	35
2	Grey fine sandy loams. Alluvial podzolics in the Kolan, Burnett, Mary and Tinana river valleys	Fairymead, Bingera, Maryborough	10:65:12:13	17
3	Fine sandy loams. Grey and red podzolics on plains and hillslopes	Millaquin, Isis, Maryborough	20:60:10:10	32
4	Fine sandy loams. Grey and red podzolics on plains and hillslopes (hard setting)	Fairymead, Bingera	24:50:13:13	17
5	Brown and grey, fine sandy to silty loams on plains and hillslopes	Moreton, Rocky Point	22:48:15:15	12
6	Black, brown, red and yellow earths. Loams to sandy clay loams on plains and hillslopes. Grey clay loams on plains and hillslopes	Bingera, Isis, Millaquin, Maryborough	20:40:10:30	21
7	Dark grey brown gleyed and alluvial clay loams and clays in the Burnett delta and coastal depressions. Black and brown alluvial clay loams	Fairymead, Bingera	15:40:20:25	15
8	Clay loams to clays on plains and hillslopes	Moreton, Rocky Point	15:25:25:35	64
9	Red kraznozems (volcanic clays). Black cracking clays on volcanic slopes	All	10:20:20:50	27

^A QDPI Bundaberg Irrigation Project, soils association map. Mary River–Tinana Creek Sugar Cane Lands, soil and land units map.

^B Particle size distributions are expressed as percentage coarse sand : fine sand : silt : clay

sugarcane soil and roots in south Queensland. Nematode populations were compared and contrasted according to geographical location, soil type, sugarcane cultivar, crop age and fallow history in order to identify the associations between these factors and the abundance of particular nematode species.

Materials and methods

Nematodes were identified and counted in root and soil samples from 240 fields, representative of all of south Queensland's sugar growing districts, from Bundaberg to the New South Wales border. All fields had grown sugarcane for more than 5 years and were sampled when plant or ratoon crops were 6–12 months old. Each sample was a composite of 10 subsamples collected from an area of 0.1–0.2 ha that was planted to a single cultivar and had a soil type of uniform appearance. Soil and roots were collected 0–30 cm from the stool, to a depth of about 30 cm. The composite sample was mixed and 2 L of soil and all the roots were retained for analysis.

Within 2 days of sampling, nematodes were extracted from 200 mL of soil using a Baermann tray (Whitehead and Hemming 1965) and from 100 ± 10 g of roots (fresh weight) using a misting cabinet (Seinhorst 1950). Nematodes were collected after 4 days of extraction and concentrated by sieving twice through a 38 µm sieve. Counts were reported as nematodes/200 mL soil or nematodes/g oven-dried root. Representative specimens were mounted on slides for confirmation of identity.

To detect root-knot nematode at low densities and provide mature females for identification, soils were also bioassayed by adding 700 mL of soil and 700 mL of pasteurised, coarse sand to a 15 cm pot. Tomato (*Lycopersicon esculentum* cv. Tiny Tim) was grown for 5 weeks and then roots were examined for galls produced by root-knot nematode. The number of galls per plant was counted. Ten mature females were retrieved from each of the bioassays of 33 representative soils and identified to species and haplotype using PCR-based diagnosis of mitochondrial DNA (Stanton *et al.* 1997).

The location of each site, sugarcane cultivar, age of crop and fallow practice used were recorded. From each sample, 300 mL of soil was air-dried and the percentages of coarse sand and fine sand were determined as the soil fractions trapped by 300 and 75 µm sieves respectively. The silt percentage and clay percentage were determined by measuring the density of a colloid suspension using a floating hydrometer at the time of mixing and after the silt had settled for 5 h (Australian Standard AS 1289.3.6.3). Soils from similar geographic locations and with similar particle size distributions were then grouped to produce 9 soil categories, as described in Table 1.

Linear relationships between nematode densities and fine soil fractions (percentage silt + clay) were evaluated using linear and polynomial regression. Differences in nematode populations associated with different biotic and abiotic factors were examined using 1-way analysis of variance (ANOVA). Where the *F*-test found significant differences at the 5% level, means were compared using the least significant difference test. The biotic and abiotic factors compared were the 9 soil type categories (Table 1),

Table 2. Nematodes detected from 240 samples from sugarcane fields in south Queensland

Means were calculated from the samples only where nematodes were detected in Baermann tray or root misting extractions

Common name	Genus and species	Sites detected (%)	Nematodes/200 mL of soil		Nematodes/g of root DW	
			Mean	Highest	Mean	Highest
Lesion	<i>Pratylenchus (P. zaeae)</i>	100	740	4480	626	4705
Spiral	<i>Helicotylenchus (H. dihystrera)</i>	87	300	4200		
	<i>Rotylenchus (R. brevicaudatus)</i>	4	82	320		
Stubby-root	<i>Paratrichodorus (P. minor, P. lobatus, P. porosus)</i>	83	145	1370		
Root-knot	<i>Meloidogyne (M. javanica, M. javanica, M. javanica, M. incognita)</i>	68	348	2040	360	6673
	<i>Tylenchorhynchus (T. annulatus, T. claytoni)</i>	68	197	2560		
Stunt	<i>Rotylenchulus (R. parvus)</i>	59	1330	12 340		
Reniform	<i>Xiphinema (X. elongatum, X. americanum)</i>	28	23	120		
Dagger	<i>X. radicumicola</i>					
	<i>Criconema (C. talanum), Criconemella (C. curvata)</i>	25	36	230		
Burrowing	<i>Ogma (O. imbricatum)</i>					
Sheath	<i>Radopholus (R. inanus)</i>	2	60	180	145	593
Needle	<i>Hemicylichophora (H. labiata)</i>	2	85	250		
Sunt	<i>Paralongidorus spp.</i>	1				
	<i>Telotylenchus spp.</i>	0.5				

5 different crop ages and fallow histories, and the 7 most common sugarcane cultivars in the region.

Since non-normal distributions are typical of nematode populations, data were transformed before analysis. Nematode densities were deemed to be adequately stabilised for ANOVA comparisons when Bartlett's test of equal variance was non-significant at the 1% level. A cube root transformation of $\sqrt[3]{(x + 0.5)}$ applied to nematodes per 200 mL of soil or per gram of oven-dried roots, was adequate for *Pratylenchus zaeae*, *Paratrichodorus* spp. and *Helicotylenchus dihystrera*, but not for *Meloidogyne* spp. and *Tylenchorhynchus annulatus*, probably because of their absence from a high proportion (32%) of the samples. For *Meloidogyne* spp. and *T. annulatus* densities, Kruskal-Wallis 1-way ANOVA was used (data were ranked irrespective of biotic and abiotic factors and a 1-way ANOVA was applied to the ranks).

Results

Species abundance

Plant parasitic nematodes were detected in every sugarcane field surveyed (Table 2). The most common species were *Pratylenchus zaeae* Graham, *Meloidogyne* spp., *Helicotylenchus dihystrera* Cobb, *Tylenchorhynchus annulatus* Cassidy, *Paratrichodorus minor* Colbran and *Rotylenchulus parvus* Sher. Criconematids and *Xiphinema* spp. were present occasionally and other genera were uncommon.

Lesion nematode was ubiquitous and *P. zaeae* was the sole species identified when specimens were compared with descriptive data on *Pratylenchus* (Frederick and Tarjan 1989).

Four *Meloidogyne* spp. in 5 haplotypes were identified (Table 3). Of those, *M. javanica* Chitwood was the dominant species, being identified in 76% of the soils that contained *Meloidogyne* spp. The bioassay detected *Meloidogyne* spp. in an additional 13% of soils where it was undetected by soil or root extractions.

The ectoparasitic nematodes identified in sugarcane soils and their abundance are presented in Table 2. The dominant stunt nematode was *T. annulatus*, whilst *Tylenchorhynchus claytoni* Steiner was identified at one site. The dominant stubby-root nematode was *P. minor* (82% of sites), followed by *Paratrichodorus lobatus* Colbran (6% of sites) and *Paratrichodorus porosus* Allen (2 sites). *Xiphinema elongatum* Schuurmans, Stekhoven and Teunissen, and *Xiphinema radicumicola* Goodey were the dagger nematodes most often

Table 3. *Meloidogyne* species identified from 33 sugarcane fields across south Queensland

Haplotype refers to a genetic class that is not necessarily related to pathogenicity

Species	Haplotype	Occurrence (%)
<i>M. javanica</i>	D	76
<i>M. arenaria</i>	A	9
<i>M. arenaria</i>	C	6
<i>M. hispanica</i>	G	6

identified, whereas *Xiphinema americanum* Cobb was identified at 1 site. *Criconeema talanum* Van den Berg and *Criconebella curvata* de Grisse and Loof were the most common ring nematodes, with *Ogma imbricatum* Colbran being identified at 3 sites.

Soil texture

The major trend in *Meloidogyne* populations was a decline in soil and root densities as percentage silt plus clay increased (Fig. 1a and b). *Meloidogyne* spp. occurred at higher ($P < 0.05$) mean densities in category 1 and 2 sands than in category 5 sandy loams and clays (category 7, 8 and 9) (Table 4). Similarly, roots from the sands contained more ($P < 0.05$) *Meloidogyne* spp. than mean root populations in some sandy loams (category 4 and 5) and clays. *Pratylenchus zaeae* occurred at a wide range of densities in all soil types. In category 8 and 9 clays, mean nematode densities in the roots were lower ($P < 0.05$) than densities in most other soil categories (Table 4), but linear regressions between nematode density and percentage silt plus clay were not significant.

Paratrichodorus spp. occurred at a wide range of densities in all soil types, but the mean density of *Paratrichodorus* spp. in category 2 and 3 sandy loams was higher ($P < 0.05$) than that in all other soil groups (Table 4). Linear regressions between nematode density and percentage silt plus clay were poor ($P < 0.05$, $R^2 = 0.25$). Mean densities of *T. annulatus* and *H. dihystra* in the soil were largely independent of percentage silt plus clay. The mean density of *H. dihystra* did not differ significantly ($P > 0.05$) between different soil categories, and mean density of *T. annulatus* was similar in most soils. Category 1 sands

and category 9 clays had lower ($P < 0.05$) mean densities of *T. annulatus* than category 2, 3, 4, 7 and 8 soils (Table 4).

Rotylenchulus parvus tended to be found at higher densities in high clay soils, but the linear relationship between nematode density and percentage silt plus clay was not significant.

Sugarcane cultivar

For most nematodes, there was no significant ($P > 0.05$) effect of sugarcane cultivar on nematode density. An exception was *P. zaeae* in roots and soil, where significantly ($P < 0.05$) lower densities were associated with cultivar CP51-21 than other cultivars (Table 5).

Crop age and fallow length

Densities of *P. zaeae* were significantly ($P < 0.05$) higher (Table 5) in the roots of plant crops than ratoon crops. Crops planted after no fallow period had similar densities of *P. zaeae* in their roots to crops planted after a 6–12 month fallow. However, densities of *P. zaeae* in the soil did not differ significantly ($P > 0.05$) between crops of different age or fallow length. Densities of *Meloidogyne* spp. were significantly ($P < 0.05$) higher (Table 5) in the roots of first ratoon crops than in third and older ratoons. However, *Meloidogyne* densities in the soil did not differ significantly ($P > 0.05$) between crops of different age or fallow length.

Densities of *Paratrichodorus* spp. around the roots of first and second ratoon crops were significantly ($P < 0.05$) higher (Table 5) than third and older ratoon crops or unfallowed plant crops. Crop age and fallow length did not significantly ($P > 0.05$) affect the population densities of either *T. annulatus* or *H. dihystra*.

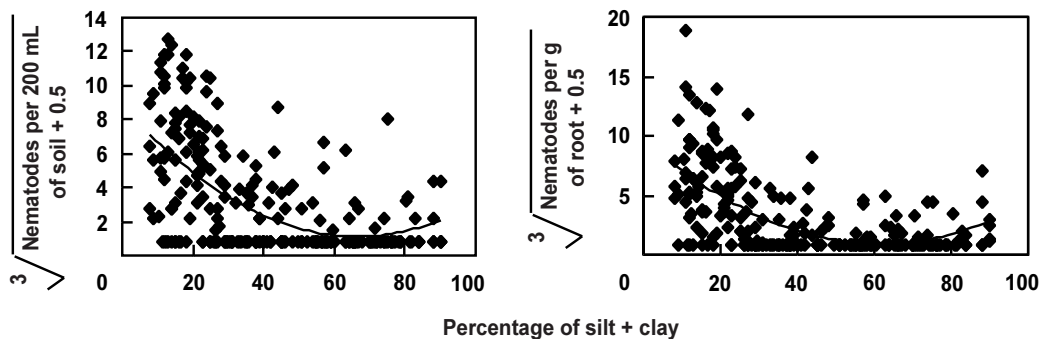


Figure 1. Polynomial regressions between the density of *Meloidogyne* spp. in (a) soil ($R^2 = 0.36$; $P < 0.05$) or (b) roots ($R^2 = 0.40$; $P < 0.05$), and soil particle size.

Table 4. Nematodes in 200 mL of soil, or per g of dry root, transformed or ranked, and compared in different soil categories

Values in the same column followed by the same letter are not significantly different at $P = 0.05$
Average I.s.d. values are shown, but exact I.s.d. values were used for pairwise testing

Soil category	<i>Pratylenchus zeae</i> in soil ^A	<i>Pratylenchus zeae</i> in roots ^A	<i>Paratrichodorus</i> spp. in soil ^A	<i>Meloidogyne</i> spp. in soil ^B	<i>Meloidogyne</i> spp. in roots ^B	<i>Tylenchorhynchus annulatus</i> in soil ^B
1	8.16bc (542)	8.99ab (726)	4.08b (68)	172a (459)	183a (658)	77b (31)
2	8.66bc (650)	8.13b (538)	5.95a (211)	181a (445)	181ab (421)	146a (207)
3	8.08bc (526)	7.64bc (445)	6.21a (239)	167ab (352)	160abc (397)	132a (121)
4	9.49ab (855)	10.42a (1130)	4.14b (70)	120abc (162)	101cd (81)	171a (479)
5	10.81a (1264)	8.49ab (612)	4.58b (96)	87c (6)	90cd (5)	132ab (135)
6	8.31bc (574)	6.27cd (246)	3.92b (60)	125abc (97)	119bcd (57)	113ab (84)
7	9.50ab (857)	8.96ab (719)	3.65bc (48)	98bc (17)	69d (12)	146a (145)
8	7.40cd (405)	5.38d (155)	2.91c (24)	74c (8)	81d (9)	135a (144)
9	6.64d (293)	5.74d (189)	2.82c (22)	95c (41)	103cd (28)	66b (23)
Average I.s.d.	1.62	1.73	1.10	53	51	61

^ATransformed to $\sqrt[3]{(x+0.5)}$. Values in parentheses are back-transformed means.

^BMean ranks from Kruskal-Wallis 1-way ANOVA. Values in parentheses are arithmetic means of the raw data.

Table 5. Nematodes in 200 mL of soil, or per gram of dry root, transformed or ranked, and compared under different cultivars, or compared in crops of different age and fallow history

Values in the same column followed by the same letter are not significantly different at $P = 0.05$
Average I.s.d. values are shown, but exact I.s.d. values were used for pairwise testing

Cultivar	<i>Pratylenchus zeae</i> in soil ^A	<i>Pratylenchus zeae</i> in roots ^A	Crop age	<i>Pratylenchus zeae</i> in roots ^A	<i>Paratrichodorus</i> spp. in soil ^A	<i>Meloidogyne</i> spp. in roots ^B
Q 124	9.24a (788)	9.05a (741)	Replant	9.69a (911)	3.02d (28)	77ab (485)
Q 136	9.86a (960)	9.07a (746)	Fallow plant	9.10a (754)	4.35bc (82)	74ab (232)
Q 137	8.85a (694)	7.75a (467)	1st ratoon	7.39b (405)	5.27a (146)	94a (346)
Q 138	8.61a (640)	7.96a (505)	2nd ratoon	6.63b (273)	4.94ab (121)	76ab (281)
Q 141	8.32a (577)	8.60a (638)	3rd ratoon +	6.48b (292)	3.65cd (49)	56b (38)
Q 146	8.58a (632)	8.89a (702)				
Q 151	7.91a (495)	8.25a (563)				
CP 51-21	6.52b (277)	5.25b (146)				
Average I.s.d.	2.10	2.28		1.64	1.11	32

^ATransformed to $\sqrt[3]{(x+0.5)}$. Values in parentheses are back-transformed means.

^BMean ranks from Kruskal-Wallis 1-way ANOVA. Values in parentheses are arithmetic means of the raw data.

Discussion

The nematodes found in the survey were typical of those found on sugarcane elsewhere. The 5 most common genera (*Pratylenchus*, *Helicotylenchus*, *Paratrichodorus*, *Meloidogyne* and *Tylenchorhynchus*) and the dominant species in those genera (*P. zaeae*, *H. dihystrera*, *P. minor*, *M. javanica* and *T. annulatus*) are widespread on sugarcane globally (Spaull and Cadet 1990). Since sugarcane in Queensland has never been systematically sampled for nematodes, a number of previously unrecorded species were found. *Meloidogyne hispanica* Hirschmann, *R. inanus* Colbran, *P. lobatus*, *T. claytoni*, *C. talanum*, *C. curvata* and *X. radicola* are first records from sugarcane in Australia as they were not listed by McLeod *et al.* (1994).

Given that a nematode's pest status depends on its abundance, its density in the field and its capacity to cause root damage, then *P. zaeae* and *M. javanica* must be considered the most important nematode pests in south Queensland canefields. *Pratylenchus zaeae* was the most widespread nematode detected, and was regularly present in the roots and soil at high densities. While *M. javanica* was not as widespread as some of the ectoparasites, its soil density was relatively high. The pathogenicities of both *P. zaeae* and *M. javanica* to sugarcane have been demonstrated in pot experiments (Harris 1974; Valle-Lamboy and Ayala 1980; Sundararaj and Mehta 1994). When nematicides have produced significant increases in sugarcane yields in Queensland (Chandler 1978; Bull 1981), Africa (Cadet and Spaull 1985) and Indonesia (Handojo *et al.* 1980), *Pratylenchus* spp. and/or *Meloidogyne* spp. were frequently the dominant nematodes involved.

The widely distributed ectoparasitic nematodes, *H. dihystrera*, *P. minor* and *T. annulatus*, were mildly pathogenic to sugarcane in glasshouse pot experiments (Apt and Koike 1962a, 1962b; Harris 1974), as more than 1000 nematodes per plant were required to affect growth. Nevertheless, the importance of ectoparasites cannot be overlooked, as their combined numbers may be sufficient to damage roots and impair root health. Due to the large size of *Xiphinema* spp. and the sluggish nature of criconematids, their recovery from soil using the Baermann tray method is poor. Thus it is expected that the incidence and soil populations of *Xiphinema* spp. and criconematids were underestimated relative to the other nematode genera. The role of these species is probably worthy of further investigation.

In south Queensland, sugarcane growth in sandy soils is commonly improved using nematicides (Bull 1979,

1981). This has resulted in the general perception within the sugar industry that nematodes are significant pests only in category 1 soils (<10% clay). Our observations are inconsistent with that perception because *P. zaeae*, *P. minor*, *T. annulatus* and *H. dihystrera* occurred at high densities across all soil types. *Meloidogyne* spp. densities were high in category 1 soils, but similar densities also occurred in category 2 and 3 soils (>10% clay) where nematicides are not routinely used. Since loam and clay soils provide a more fertile environment for sugarcane growth than sandy soils, crop loss due to nematodes is likely to be lower (Donaldson 1985). Therefore, sugarcane growers on loam and clay soils are likely to dismiss nematodes as pests due to the apparent health of the crop, whereas subtle but significant yield losses could be occurring. Also, it is difficult to obtain responses from nematicides because they tend to be adsorbed onto clay particles and organic matter (Abdellatif *et al.* 1967; Awad *et al.* 1984). Thus, nematode problems may be more widespread than has been thought in the past.

The lower densities of *P. zaeae* associated with cultivar CP51-21 than other cultivars may be due to CP51-21 having some nematode resistance. Alternatively, this cultivar may possess a lower rooting density than other cultivars, thereby limiting the habitat available for *P. zaeae*. Further studies are warranted to investigate possible cultivar resistance to *P. zaeae*.

Fallowing had no more than a short-term effect on nematode populations, as evidenced by the high densities of *P. zaeae*, *Meloidogyne* spp., *T. annulatus* and *H. dihystrera* on plant crops. Clearly, nematodes are being maintained in the soil between successive crop cycles, probably because the fallow periods are short (2–10 months) and are often infested by host weeds. Vigorous root growth by newly planted crops probably allows the development of the high nematode densities, as observed. Less vigorous root growth is generally found in third ratoon and older crops and perhaps this lack of a food source is the reason that *P. zaeae* and *Paratrichodorus* spp. were found at low densities on those crops. Alternatively, natural enemies of these nematodes may take several years to build up to suppressive levels, as has been observed for nematodes on other perennial crops (Stirling 1991).

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