PLANT-PARASITIC AND FREE-LIVING NEMATODES ASSOCIATED WITH SUGARCANE IN NORTH QUEENSLAND

By

J BULL[1], GR STIRLING[2], R MAGAREY[1]

[1] *Sugar Research Australia, Tully*
[2]*Biological Crop Protection Pty Ltd, Brisbane*
graham.stirling@biolcrop.com.au

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Abstract

SURVEYS OF NORTH Queensland sugarcane soils indicated that root-lesion nematode (*Pratylenchus zeae*) was the most important nematode pest because it occurred in most fields and was often present at high population densities. However, the presence of root-knot nematode (*Meloidogyne* spp.) in sandy soils and the relatively widespread distribution of moderately pathogenic nematodes such as stunt nematode (*Tylenchorhynchus annulatus*), stubby root nematode (*Paratrichodorus minor*), ring nematode (various Criconematidae) and dagger nematode (*Xiphinema elongatum*) suggested that in most fields, several nematode species were feeding on roots and contributing to root health problems. With regard to the free-living nematode community, survey results showed that bacterial-feeding nematodes were much more common than fungal-feeding nematodes and numbers of omnivorous and predatory nematodes were relatively low. Collectively, these findings suggest that the biological status of many north Queensland sugarcane soils is relatively poor. In addition to having high numbers of plant-parasitic nematodes they have low numbers of fungal-feeding nematodes. This indicates that fungi are not an important component of the detritus food web and this has implications for the ecosystem services they provide: decomposition of recalcitrant forms of organic matter; aggregation of soil particles; enhancement of plant nutrient uptake; improvement of disease resistance in plants; and suppression of pests and pathogens. The lack of omnivorous and predatory nematodes also has implications for pest suppression, as these nematodes help regulate populations of plant-parasitic nematodes.

Introduction

Plant-parasitic nematodes are important pests of sugarcane worldwide, with most cane fields infested with at least five pest species (Cadet and Spaull, 2005). In Australia, estimates obtained from nematicide trials suggest that nematodes are one of the sugar industry's most important soilborne pests and are costing growers more than $80 million in lost production (Blair and Stirling, 2007).

Previous surveys for these pests have focused on the Burdekin, central and southern cane-growing regions (Blair *et al*., 1999 a, b). Consequently, there is no published information on the occurrence and distribution of plant-parasitic nematodes in north Queensland, a region that consists of seven mill districts (from Ingham to Mossman) and covers about 38% of the sugarcane grown in Queensland.

This paper provides data on the occurrence of plant-parasitic nematodes in north Queensland sugarcane soils. The data were obtained from three sources:

1) analyses of nematode diagnostic samples forwarded to Sugar Research Australia in the five years from 2012 to 2016;
2) nematodes found in a survey to determine whether *Pasteuria*, a bacterial biocontrol agent of nematodes, was present in north Queensland sugarcane fields; and
3) a survey in north Queensland that aimed to determine whether root health and suppressiveness to nematode pests varied with depth in the soil profile, as has been observed in the Bundaberg region of Queensland (Stirling *et al*., 2011).

Observations on the free-living nematode community are also included because this group of nematodes help suppress soil-borne pests and diseases (Stirling, 2014) and are useful environmental indicators (Stirling *et al*., 2007; Wilson and Kakouli-Duarte, 2009).

Materials and methods

Nematodes in diagnostic samples

The nematode diagnostic service provided by Sugar Research Australia processes soil and root samples collected from trial sites and soil samples forwarded by growers and consultants. The database for the five years from 2012 to 2016 was searched for results from north Queensland and after trial samples, pre-plant samples and samples collected in the plant crop were discarded, a total of 93 samples remained.

Results from those samples are reported in this paper. Instructions for collecting the samples state that in each block of sugarcane, 12–15 cores should be collected at random from the edge of the row to a depth of 25 cm and a 500 g sub-sample forwarded to the laboratory for analysis. We have assumed that most of the samples were collected in this way.

The first author processed the samples by spreading a weighed sample of soil (200 g dry weight equivalent) on a tray, incubating the samples for 4 days at 22–28 °C and then retrieving the nematodes on a 38 µm-aperture sieve (Whitehead and Hemming, 1965).

Plant-parasitic nematodes were counted by genus while free-living nematodes were subdivided into bacterivores (mainly nematodes in the Rhabditidae and Cepahalobidae, but also genera such as *Plectus*, *Geomonhystera*, *Rhabdolaimus*, *Prismatolaimus*, *Teratocephalus* and *Alaimus*); fungivores (*Aphelenchus* and *Aphelenchoides*); plant associates (fine-tailed Tylenchidae); omnivores (Dorylaimida); and predators in the Order Mononchida. Numbers of bacterivores (B) and fungivores (F) were used to calculate the Nematode Channel Ratio [B/(B+F)], which is an indicator of whether the main decomposition channel in the detritus food web is dominated by bacteria or fungi (Yeates 2003).

A survey of nematodes in north Queensland

Information on the distribution of *Pasteuria* in Queensland sugarcane fields has recently been published (Stirling *et al*., 2017) and in one component of that survey, 14 soil samples were collected in October 2014 from the two mill districts in the Herbert region. An additional set of 25 samples was collected in October 2016 from the five cane-growing regions in tropical north Queensland (around Mossman, Cairns, Innisfail and Tully, and on the Atherton Tableland).

The fields selected were all in the latter stages of the crop cycle (fourth or later ratoons) and each sample was a composite of about 15 sub-samples taken at a depth of 0–15 cm. Samples were processed by the second author using the method described previously, except that nematodes were retrieved after 2 days by sieving twice on a sieve with an aperture of 38 µm. In addition to checking for nematodes encumbered with *Pasteuria* endospores, plant-parasitic and free-living nematodes were counted and the latter group was also differentiated into microbial feeders, omnivores and predators.

Nematode distribution with depth

In June 2014, soil samples were obtained from 11 fields in north Queensland. All fields had clay loam or clay soils and contained second, third or fourth ratoon crops of sugarcane variety Q208⟨D⟩. At each site, soil was collected at depths of 0–5, 5–10 and 10–15 cm from a 15 × 15 cm site in the middle of a cane row. Thus, a total of 33 samples (11 sites × 3 depths) were collected and they were processed as described in the previous paragraph. Additionally, root biomass was measured and the roots in each sample were rated for the presence and functionality of fine roots using the following scale: 1= severely diseased, with no fine roots; 2= highly diseased, with fine roots erratically distributed and contributing <20% of total root length; 3= intermediate levels of disease, with fine roots contributing 20–50% of total root length; 4= mainly healthy, with large numbers of functional fine roots; 5= very healthy, with a uniform spread of functional fine roots contributing >90% of total root length (Stirling *et al*., 2011).

Results

Nematodes in diagnostic samples

Seven plant-parasitic nematodes were detected in the samples, with *Pratylenchus* and *Tylenchorhynchus* the most commonly-occurring genera (Table 1). *Pratylenchus* was detected in all but one of the samples and its population density was consistently high. One field had a count of almost 5 000 *Pratylenchus*/200 g soil and 30% of the fields had counts >1 000.

Counts of free-living nematodes were quite variable, as some samples had <100 nematodes/200 g soil and others had counts >5 000. In general, numbers were relatively low, as 34% of samples had <1 000 free-living nematodes/200 g soil and counts for 39% of the samples ranged from 1 000 to 3 000. The Nematode Channel Ratio was greater than 0.9 in one-third of the samples and the mean ratio was 0.8, indicating that the microbivorous nematode community in most soils was dominated by bacterivores. In samples with more than 500 free-living nematodes/g soil, an average of 5% and 3% of the nematodes were omnivores and predators, respectively. Plant associates were much more common as 11% of the free-living nematodes were in this group. However, they sometimes comprised 20–40% of the nematode community.

Table 1—Occurrence and population densities of plant-parasitic nematodes (averaged across the fields in which the nematodes were detected) in 93 diagnostic samples from sugarcane fields in north Queensland.

Common name	Genus	Occurrence (% of fields)	Nematodes/200 g soil	
			Mean	Highest count
Root-lesion	*Pratylenchus*	99	942	4981
Spiral	*Helicotylenchus*	75	317	1669
Stubby	*Paratrichodorus*	43	106	613
Ring	Family Criconematidae	40	71	418
Stunt	*Tylenchorhynchus*	84	281	2156
Root-knot	*Meloidogyne*	47	235	1896
Dagger	*Xiphinema*	24	43	152

A survey of nematodes in north Queensland

The plant-parasitic nematodes detected in samples from the Herbert and tropical north Queensland are listed in Table 2. The nematodes present were similar to those obtained in the diagnostic samples but the results for the two most important nematode pests of sugarcane deserve some comment.

Root-lesion nematode was ubiquitous and its population densities were sometimes extremely high, with two fields near Cairns having population densities greater than 5 000/200 g soil. Root-knot nematode was not as widespread, largely because many of the samples came from heavy-textured soils.

Nevertheless, it was relatively common in sandy loam soils and was present at high population densities (>1 000 nematodes/200 g soil) at two sites in the Herbert and one in the Mossman mill district.

Although the survey covered only a limited number of fields, there appeared to be differences in the range of plant-parasitic nematodes present in the northern and southern part of the region. A pin nematode was reasonably common in the Herbert but was not detected in the north, while *Rotylenchulus parvus* and *Achlysiella williamsi* were only detected in the north.

Populations of free-living nematodes were relatively high in both parts of the region, but this component of the nematode community was primarily composed of bacterivores and fungivores. Populations of omnivores were usually very low but reasonable numbers of predators in the family Mononchidae were sometimes observed.

These predatory nematodes were most common in the Tully and Innisfail mill districts where they sometimes accounted for 5–15% of the nematode community.

Table 2—Occurrence and population densities of plant-parasitic and free-living nematodes (averaged across the fields in which the nematodes were detected) in 39 north Queensland sugarcane fields (25 in the northern part of the region and 14 in the Herbert).

Common name	Scientific name	Occurrence (% of fields)		Nematodes/200 g soil			
				Mean		Highest count	
		North	Herbert	North	Herbert	North	Herbert
Root-lesion	*Pratylenchus zeae*	100	100	853	316	6500	940
Spiral	*Helicotylenchus dihystera*	84	88	163	118	1299	370
Stubby	*Paratrichodorus minor*	32	41	33	69	160	180
Ring	Family Criconematidae	76	41	46	25	192	64
Stunt	*Tylenchorhynchus annulatus*	44	76	145	85	481	370
Root-knot	*Meloidogyne* spp.	40	47	229	502	1225	1920
Dagger	*Xiphinema elongatum*	52	24	49	26	263	42
Reniform	*Rotylenchulus parvus*	8		960		1041	
–	*Achlysiella williamsi*	8		41		46	
Pin	*Gracilacus* sp.		59		177		750
Free-living		100	100	2786	5409	11520	13350

Nematode distribution with depth

Observations of the roots collected from 11 fields at three different depths in the profile showed that the fine root rating was always highest in the surface soil and declined with depth. Fine root ratings (averaged across all fields and depths) were 3.4, 1.0 and 1.1 at 0–5, 5–10 and 10–15 cm, respectively.

Eight plant-parasitic nematodes were detected in the samples but root-lesion nematode (*Pratylenchus zeae*) was the only nematode present in every field (Table 3). Spiral and ring nematodes were also relatively common, as they were found in 9 of the 11 fields sampled. Root-lesion nematode was the dominant plant parasite as its population densities at all depths were usually higher than the other nematodes. The high numbers of spiral nematode, as recorded in Table 3 at a depth of 0–5 cm, is somewhat misleading, as this figure was magnified by an extremely high count (more than 12 000 nematodes/200 g soil) at one site on the Atherton Tableland.

Numbers of free-living nematodes were consistently higher in surface soils than at depth (Table 3). However, almost all of these nematodes were microbivores and plant associates. Numbers of omnivorous and predatory nematodes were on average less than 4% of the nematode community, regardless of depth.

Table 3—Mean population densities of free-living and plant-parasitic nematodes at three depths (averaged across the fields in which the nematodes were detected) in 11 north Queensland sugarcane fields.

Common name	Scientific name	Occurrence (% of fields)	Nematodes/200 g soil		
			0–5 cm	5–10 cm	10–15 cm
Root-lesion	*Pratylenchus zeae*	100	625	920	888
Spiral	*Helicotylenchus dihystera*	82	1944	357	244
Ring	Family Criconematidae	82	376	170	101
Stubby	*Paratrichodorus minor*	45	334	351	241
Stunt	*Tylenchorhynchus annulatus*	45	91	247	119
Dagger	*Xiphinema elongatum*	55	39	17	20
Reniform	*Rotylenchulus parvus*	18		48	202
Root-knot	*Meloidogyne* spp.	9	57		
Free-living	–	100	3657	1548	1262

Discussion

The results presented in Tables 1–3 indicate that most of the nematode pests found on sugarcane in southern and central Queensland by Blair *et al*. (1999 a, b) also occur in north Queensland. However, our results also showed that some plant-parasitic nematodes recorded in the southern surveys were either absent or not widely distributed in north Queensland.

Reniform nematode (*Rotylenchulus parvus*) and one of the spiral nematodes (*Rotylenchus brevicaudatus*) are relatively common in some southern regions (e.g. Bundaberg) but were either not observed or rarely seen in the surveys reported here.

Pratylenchus zeae is clearly the most important nematode pest in north Queensland as it was detected in nearly every field and was often present at high population densities. However, the presence of root-knot nematode in sandy soils and the relatively widespread distribution of moderately pathogenic nematodes (e.g. stunt, stubby, ring and dagger nematode) suggests that in most cane fields in north Queensland, several nematode species are feeding on roots and contributing to root health problems.

We found one site on the Atherton Tableland with more than 12 000 spiral nematodes (*Helicotylenchus dihystera*)/200 g soil at a depth of 0–5 cm. However, this is not necessarily a concern as this species is relatively non-pathogenic and high population densities tend to be associated with high-yielding cane (Cadet *et al*., 2002).

Although the results presented in Tables 2 and 3 were obtained from only 50 fields, the much more frequent occurrence of ring nematode in north Queensland compared with southern cane-growing regions raises questions about its importance in the tropics.

These highly annulated nematodes are very slow-moving and are not readily extracted with the tray method used in these studies.

If population densities had been measured with a method capable of extracting inactive nematodes from soil (e.g. a centrifugal floatation method), the ring nematode count is likely to have been 5–15 times higher. Given that very high numbers of ring nematode are likely to occur in some north Queensland cane fields, further research on this group of nematodes should be undertaken.

Populations must be measured using appropriate extraction techniques and the pathogenicity of each species determined. Blair *et al*. (1999 a, b) found five criconematids in southern and central regions (*Criconema talanum*, *C. xenoplax*, *C. caelata*, *Criconemella curvata* and *Ogma imbricatum*) but taxonomic studies are required to determine whether the same species mix occurs in north Queensland.

The presence of *Achlysiella* in one of our samples also raises questions about the distribution of this species. *A. williamsi* was known to be present on sugarcane in north Queensland in the 1980s (McLeod *et al.* 1994; KJ Chandler, personal communication) and has also been found in Mackay (McLeod *et al*., 1994). However, its distribution in Queensland has never been determined. Since it occurs on sugarcane in New Guinea (Hunt *et al*., 1989), it is possible that the tropical environment in north Queensland is ideal for this nematode.

Rotylenchulus reniformis is another plant-parasitic nematode on which further work is justified. Although it was not detected in the surveys reported here, this reniform nematode species was found by the second author at a relatively high population density (2 600 nematodes per 200 g soil) in a diagnostic sample from a field north of Cairns that had recently been fallowed following sugarcane.

Although it is related to *R. parvus*, a relatively non-pathogenic species that commonly occurs on sugarcane, *R. reniformis* is likely to be more damaging. It is a serious pest of pineapple, cotton, sweet potato and many other crops in tropical regions of the world and since sugarcane is a known host (MacGowan, 1977), its distribution and pathogenicity on sugarcane should be determined.

In the survey in which soil was sampled at three different depths, root health was assessed using a fine-root rating. Those results indicated that root health was poor, particularly in the 5–15 cm zone of the soil profile. Since populations of *P. zeae* at this depth were consistently high, this nematode would have been partly responsible for the damage observed. However, other root pathogens, particularly those that are active in wet soils, are also likely to have been contributing because samples were collected after 8 weeks of consistently wet weather.

Results from the depth survey also showed that numbers of free-living nematodes were high in the surface soil and declined with depth. This depth distribution is commonly observed in sugarcane soils (Stirling, 2017) and reflects the fact that the biomass of the microbes consumed by bacterial and fungal-feeding nematodes is influenced by soil carbon levels that vary with depth.

Since microbivorous nematodes mineralise nutrients from the bacteria and fungi they use as a food source (Ingham *et al*., 1985; Ferris *et al*., 1998; Chen and Ferris, 1999), their presence in surface soils is one of the reasons that root health is better than further down the profile.

When the composition of the free-living nematode community is considered, two important observations were made:

1) bacterial-feeding nematodes were much more common than fungal-feeding nematodes; and
2) numbers of omnivorous and predatory nematodes were relatively low.

Both of these findings suggest that from a biological perspective, many north Queensland sugarcane soils are in poor condition. Low numbers of fungal-feeding nematodes indicate that fungi are no longer an important component of the detritus food web and this has implications for the ecosystem services they provide: decomposition of recalcitrant forms of organic matter; aggregation of soil particles; enhancement of plant nutrient uptake; improvement of disease resistance in plants; and suppression of pests and pathogens (Stirling, 2014; Stirling *et al*., 2016). The lack of omnivorous and predatory nematodes also has implications for pest suppression, as these nematodes help regulate populations of plant-parasitic nematodes (Yeates and Wardle, 1996).

Collectively, the results presented in this paper suggest that the biological health of north Queensland sugarcane soils is relatively poor. That conclusion is based on the fact that in most soils, populations of plant-parasitic nematodes were relatively high while the free-living nematode community was dominated by bacterial-feeding nematodes and contained relatively few omnivorous and predatory nematodes. Unpublished results from the four north Queensland sites included in a study that compared cane-growing soils with soils under grass pasture (Stirling *et al*., 2010) also indicated that the biological health of sugarcane soils was much worse than adjacent soils under pasture.

Minimising tillage, reducing compaction, maintaining a permanent cover of crop residues, reducing pesticide inputs and improving nitrogen management may improve the situation (Stirling *et al*., 2016) but ultimately, the only way to obtain a balanced nematode community is to increase levels of soil organic matter and restore the suppressive services that have been lost from sugarcane soils.

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