

NEMATODES ASSOCIATED WITH SUGARCANE (*SACCHARUM* SPP.) IN SANDY SOILS IN PARANÁ, BRAZIL

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ABSTRACT

Severino, J. J., C. R. Dias-Arieira, and D. J. Tessmann. 2010. Nematodes associated with sugarcane in sandy soils in Paraná, Brazil. *Nematropica* 40:111-119.

The aim of this study was to identify and quantify phytonematodes in sugarcane cultivated in sandy soils in ten municipalities in northwestern Paraná state, Brazil. In total, 74 samples of soil and roots in the rhizosphere of sugarcane were collected. In roots, the most frequently observed genus was *Meloidogyne*, found in 96.8% of the samples, followed by *Pratylenchus* (85.3%), and *Helicotylenchus* (31.3%). In soil, the most common genus was *Pratylenchus*, found in 94.3% of the samples, followed by *Meloidogyne*, *Helicotylenchus* and *Xiphinema*. Trichodoridae (*Paratrichodorus* and *Trichodorus*), *Aphelenchus*, *Hoplolaimus*, *Mesocriconema* and *Hemicycliophora* were less common. *Pratylenchus zaei* was present in 73% of the material examined and *P. brachyurus* in 13.5%. *Helicotylenchus dihystera* was found in 68.8% of the samples and was the only species within this genus identified.

Key words: Field survey, *Helicotylenchus*, nematodes, *Pratylenchus*, *Saccharum*.

RESUMO

Severino, J. J., C. R. Dias-Arieira, and D. J. Tessmann. 2010. Nematóides associados à cana-de-açúcar em solos arenosos do Paraná, Brasil. *Nematropica* 40:111-119.

Com o objetivo de identificar e quantificar os fitonematóides em cana-de-açúcar cultivada em solos arenosos do Paraná, Brasil, foi conduzido um levantamento compreendendo dez municípios da região Noroeste do Paraná. Foram coletadas 74 amostras de 1,5 kg de solo e raízes na rizosfera de cana-de-açúcar. O processamento das amostras ocorreu utilizando 100 cm³ de solo e 10 g de raízes através da técnica de flotação centrífuga em solução de sacarose. Após o processamento, as amostras foram analisadas em lâmina de Peters sob microscópio óptico ou em lâminas temporárias, com auxílio de chaves taxonômicas. Em raízes, ao nível de gênero, a maior frequência observada foi para *Meloidogyne*, presente em 96,8% das amostras, seguido por *Pratylenchus*, 85,3%, e *Helicotylenchus*, 31,3%. No solo, a maior frequência observada foi para *Pratylenchus*, encontrado em 94,3% das amostras, seguido de *Meloidogyne*, *Helicotylenchus* e *Xiphinema*. Tricodoridaes (*Paratrichodorus* e *Trichodorus*), *Aphelenchus*, *Hoplolaimus*, *Mesocriconema* e *Hemicycliophora* foram encontrados em menores frequências. Quanto às espécies, *Pratylenchus zaei* foi constatada em 73% do material avaliado e *P. brachyurus* em 13,5%. *Helicotylenchus dihystera* foi observada em 68,8% das amostras, sendo esta, a única espécie dentro deste gênero identificada no levantamento.

Palavras-chave: *Helicotylenchus*, Levantamento, Nematóides, *Pratylenchus*, *Saccharum*.

INTRODUCTION

Sugarcane is a major agricultural crop produced in many countries with tropical

and subtropical climates. It is largely used in the food industry, as well as to produce fibers and biofuel. However, because it is a monoculture and in many countries is

grown in degraded soils with high levels of sand, problems caused by phytonematodes are common. In Brazil, this crop occupies an area superior to seven millions of hectares, with yield estimated in more than 610 millions of tons, expecting increase to the next agriculture year (Conab, 2010). Medium longevity of sugarcane crop in sandy soils is about five years, reaching to eight years, according to adopted management practice.

Over 310 species from 48 genera of endo- and ecto-parasitic nematodes associated with rhizosphere soil and roots have been verified in sugarcane (Cadet and Spaul, 2005). No recent data are available regarding damage caused by these pathogens to this crop, but it is known that controlling them raises yields by between 8% and 40%, depending on the species of nematode and hybrid planted (Dinardo-Miranda *et al.*, 1995; Dinardo-Miranda, 2001). In general, a diverse range of nematodes is associated with sugarcane rhizosphere. The sedentary endoparasites of the genus *Meloidogyne* spp. (Starr and Bendezu, 2002) and migrating endoparasites of the genus *Pratylenchus*, particularly *Pratylenchus zae* (Moura *et al.*, 1990), are considered the most economically important.

The pathogenicity of ectoparasites in Brazil has not yet been thoroughly researched, although there are studies showing almost permanent associations between sugarcane crops and some nematodes, such as *Helicotylenchus*, *Paratrichodorus*, *Trichodorus*, *Tylenchorhynchus*, *Hemicycliophora*, *Xiphinema* and *Mesocriconema* (Moura, 2000). In Brazil, the first citation of parasitic nematodes in sugarcane took place in the state of São Paulo, in 1962, when the genera *Helicotylenchus* and *Trichodorus* were recorded in sugarcane variety Co290 (Brieger, 1962). The first survey of phytonematodes in sugarcane in Brazil was carried out between October

1970 and April 1973 in the state of São Paulo, where the genus *Helicotylenchus* was found in more than 90% of the 800 samples collected (Novaretti *et al.*, 1974). In the same survey, the genera *Meloidogyne*, *Pratylenchus*, *Xiphinema*, *Hoplolaimus*, *Trichodorus*, *Tylenchorhynchus*, *Tylenchus*, *Peltamigratus*, *Aphelenchoides*, *Aphelenchus*, *Mesocriconema*, *Ditylenchus*, *Helicotylenchus*, *Hemicycliophora*, *Hexatylus* and *Anguina* were identified.

Identifying nematodes in crop areas is the first step to determine if nematode management is needed, influencing the choice of crop varieties, that may show variable behavior in relation to nematodes populations (Novaretti *et al.*, 1988; Dinardo-Miranda *et al.*, 1995). Therefore, considering the importance of sugarcane and its increasing cultivation in the state of Paraná, as well as the lack of research on nematodes in the region, the aim of this study was: (i) to conduct a survey on the presence of phytonematodes in sugarcane crops in areas of sandy soils in northwestern Paraná; (ii) to quantify the genera of plant-parasitic nematodes; and (iii) to identify species of *Pratylenchus* and *Helicotylenchus*.

MATERIALS AND METHODS

Seventy four samples each containing 1.5 kg of soil and sugarcane roots were collected, between October, 2005 to April, 2006 from sandy soils in sugarcane crop areas in the Arenito Caiuá region of northwestern Paraná, Brazil (Fig. 1). This area comprises the municipalities of Cidade Gaúcha, Maria Helena, Tapira, Tapejara, Cianorte, São Tomé, Cruzeiro do Oeste, Alto Piquiri, Perobal and Umuarama. Areas were randomly selected in areas with a history of at least 15 years of sugarcane crop production. Plants sampled were between the second and eighth ratoon in the crop production cycle.

Each composite sample was made up of between 15 and 20 simple samples depending on the size of the plot, with an average plot size of 15 ha, in depth of 20 cm. Areas featuring high weed concentrations, areas next to terraces, and those containing limestone deposits, organic matter, and water features were avoided for sample collection. The samples were placed in plastic bags labeled with information on the area, variety, date and name of farm, and sent to the Phytopathology Laboratory of the State University of Maringá to be processed.

To extract nematodes from the soil, 100 cm³ was processed using the centrifugal flotation technique in sucrose solution and the samples were then stored in labeled containers. For the roots, 10 grams was processed using a method adapted by Boneti and Ferraz (1981). After extraction, the samples were immersed in 60°C water for five minutes, and 10 drops of 40% formalin were added to the solution in order to kill and preserve the nematodes (Tihohod, 1997).

The nematodes were identified and quantified with the aid of Peter's counting slides under an optical dissecting microscope. Permanent slides were prepared for observations at magnifications of 400 or 1,000x. To identify genera, the taxonomic keys used were Mai and Lyon (1975), Tarjan 1990, Nickle 1991 and Tihohod 1997.

To identify species of *Pratylenchus* and *Helicotylenchus*, temporary slides were prepared with a technique described by Tihohod (1997). Briefly, measurements were taken using a micrometric lens attached to the objective lens. Ten females of *Pratylenchus* from each sample were examined under a light microscope. Measurements were taken of body length, maximum body width, distance between lip region and the vulva, body width at the vulva, stylet length, width and height of stylet knobs, tail length, tail shape and V% (distance

between lip region and vulva X 100, divided by body length), according to parameters proposed by Frederick and Tarjan (1989), Nickle (1991) and Troccoli *et al.* (1996).

For *Helicotylenchus*, measurements were taken of maximum body width, stylet length, width and height of stylet knobs, body width at the anus and tail length, according to Fortuner (1984) and Wouts and Yeates (1994).

RESULTS

In the sampled areas, the predominant varieties of sugarcane were RB72454, RB835486 and RB845210, representing 46%, 14% and 11% of all varieties, respectively. The other varieties sampled were RB867515, RB855113, SP801842, RB845089, RB835054, RB845057, RB845239 and PO8862.

In the 74 root samples analyzed, the genera *Meloidogyne* and *Pratylenchus* were found in 96.8% and 85.3% of samples, respectively. The genus *Meloidogyne* was the most abundant in all regions except the municipalities of Tapejara and Cruzeiro do Oeste, where *Pratylenchus* was the predominant genus (Table 1). In terms of the number of nematodes present in the samples, the highest populations of *Pratylenchus* were found in the municipalities of Tapejara and Cruzeiro do Oeste, with respective averages of 329.0 and 328.3 specimens in 10g of roots. The highest *Meloidogyne* populations were found in the municipality of Umuarama, with an average of 465.1 juvenile specimens in 10g of roots (Table 1).

In the soil samples, the average frequencies of the genera *Meloidogyne* and *Pratylenchus* were 66.0% and 94.3%, respectively (Table 2). *Helicotylenchus* and *Xiphinema* were found in 82.9% and 54.7% of the samples analyzed, respectively. In the municipalities of Maria Helena, Tapejara

Table 1. Frequency and average number of *Meloidogyne*, *Pratylenchus* and *Helicotylenchus* in 10 g of sugarcane roots obtained from sandy soils in northwestern Paraná, Brazil.

Municipality/ Field sampled	Frequency (%)			Average number		
	<i>Meloidogyne</i>	<i>Pratylenchus</i>	<i>Helicotylenchus</i>	<i>Meloidogyne</i>	<i>Pratylenchus</i>	<i>Helicotylenchus</i>
Gianorte (25)	88.0	96.0	8.0	108.2	83.8	0.7
São Tomé (17)	94.1	88.2	5.9	144.4	70.2	0.5
C. Gaúcha (6)	100.0	100.0	50.0	183.7	87.3	10.8
Tapira (2)	100.0	100.0	100.0	106.0	76.5	22.5
M. Helena (1)	100.0	100.0	100.0	185.0	93.0	24.0
Tapejara (1)	100.0	100.0	0.0	132.0	329.0	0.0
Umuarama (7)	100.0	57.1	28.6	465.1	169.9	6.0
Perobal (5)	100.0	60.0	20.0	247.2	62.4	4.6
A. Piquiri (3)	100.0	66.6	0.0	186.7	101.3	0.0
C. Oeste (7)	85.7	85.7	0.0	211.9	328.3	0.0

Table 2. Frequency and average number of nematodes in 100 cm³ of soil collected in the rhizosphere of sugarcane in sandy soils in northwestern Paraná, Brazil.

Municipality/ Field sampled	Frequency of nematodes in the soil (%)									
	Meloid. ¹	Pratyl. ²	Helic. ³	Xipl. ⁴	Tricod. ⁵	Hopl. ⁶	Mesoc. ⁷	Hemic. ⁸	Aphel. ⁹	
Cianorte (25)	56.0	96.0	44.0	16.0	0.0	4.0	8.0	0.0	0.0	
São Tomé (17)	64.7	100.0	47.0	47.0	2.9	11.7	0.0	0.0	0.0	
C. Gaúcha (6)	50.0	100.0	66.6	66.6	16.6	0.0	0.0	0.0	0.0	
Tapira (2)	0.0	100.0	100.0	100.0	0.0	0.0	0.0	0.0	0.0	
M. Helena (1)	100.0	100.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	
Tapejara (1)	100.0	100.0	100.0	0.0	50.0	0.0	0.0	0.0	0.0	
Umuarama (7)	100.0	100.0	100.0	71.4	42.8	0.0	28.6	0.0	85.7	
Perobal (5)	80.0	80.0	100.0	60.0	5.0	40.0	0.0	20.0	80.0	
A. Piquiri (3)	66.6	66.6	100.0	100.0	16.6	66.6	0.0	0.0	66.6	
C. Oeste (7)	42.8	100.0	71.4	85.7	14.3	0.0	0.0	0.0	0.0	
Average	66.0	94.3	82.9	54.7	14.8	12.2	3.7	2.0	23.2	
Average number of nematodes in the soil (%)										
Cianorte (25)	16.8	68.8	14.1	3.4	0.0	0.8	1.1	0.0	0.0	
São Tomé (17)	26.4	123.4	14.5	1.2	0.6	3.8	0.0	0.0	0.0	
C. Gaúcha (6)	82.0	113.7	23.8	19.7	4.0	0.0	0.0	0.0	0.0	
Tapira (2)	0.0	119.5	48.0	0.0	0.0	0.0	0.0	0.0	0.0	
M. Helena (1)	118.0	165.0	48.0	0.0	0.0	0.0	0.0	0.0	0.0	
Tapejara (1)	146.0	73.0	122.0	0.0	36.5	0.0	0.0	0.0	0.0	
Umuarama (7)	150.1	115.3	112.6	36.3	23.2	0.0	8.3	0.0	34.6	
Perobal (5)	150.0	162.0	91.0	28.4	24.2	8.0	0.0	4.6	26.8	
A. Piquiri (3)	98.3	77.7	114.3	28.3	2.8	26.3	0.0	0.0	30.7	
C. Oeste (7)	109.0	273.9	16.4	43.6	3.5	0.0	0.0	0.0	0.0	

¹Meloidogynae; ²Pratylenchus; ³Helicotylenchus; ⁴Xiphinema; ⁵Trichodoridae; ⁶Hoplolaimus; ⁷Mesocriconema; ⁸Hemictylophora; ⁹Aphelenchus.

and Umuarama, *Meloidogyne* and *Pratylenchus* were present in 100% of the soil samples, while in Cianorte, the respective frequencies were 56% and 96%. In Perobal, 80% of the samples contained *Meloidogyne* and *Pratylenchus*, and in Alto Piquiri, these nematodes were in 66.6% of the samples. In São Tomé, Cidade Gaúcha, Tapira and Cruzeiro do Oeste, *Meloidogyne* was detected in 64.7%, 50.0% and 42.8% of the samples, while *Pratylenchus* was observed in 100% of the soil samples taken from these municipalities. The municipality of Cruzeiro do Oeste presented the highest number of *Meloidogyne*, with 255 specimens in 100 cm³ of soil, followed by Perobal, with 187, and Cidade Gaúcha, with 164. For the genus *Pratylenchus*, the samples collected in the municipality of Cruzeiro do Oeste also presented the highest population, with 274 specimens, followed by Perobal and Maria Helena, with 203 and 165, respectively.

Pratylenchus zaeae was identified in 73.0% of all soil samples, and *P. brachyurus* in 13.5%. In 9.5% of the samples it was not possible to identify the specific species, and in 4.0% of the samples *Pratylenchus* species were absent. Morphometric data on the *Pratylenchus* specimens in the different municipalities were confirmed to relate to *P. zaeae*, since the 10 female specimens analyzed all presented bodies ranging from 420.2 to 660.4 µm in length, with an average of 541.1 µm. Stylet length varied from 15.5 to 17.6 µm, with an average of 16.5 µm. In terms of V%, the variation was between 63.1 and 75.8, with an average of 69.3%. *Pratylenchus brachyurus* presented body lengths of between 490.1 and 712.1 µm, with an average of 638.5 µm, and stylet length of between 16.1 and 21.4 µm, with an average of 19.9 µm; V% varied from 82.1 to 88.0, with an average of 85.3.

The genus *Helicotylenchus* was found in 82.9% of the soil samples analyzed; in 68.8% of all samples, the species was identi-

fied as *H. dihystra*. The morphometric data on *Helicotylenchus* presented a stylet length of 25.0 to 27.0 µm, with an average of 26.30 µm. Maximum body width varied from 20.0 to 24.2 µm, with an average of 22.58 µm, and stylet knob width ranged between 5.1 and 5.8, with an average of 5.50 µm.

Other nematodes species were found in the soil, such as *Helicotylenchus*, *Xiphinema*, *Hoplolaimus*, *Trichodorus*, *Paratrichodorus*, *Mesocriconema*, *Aphelenchus* and *Hemicyclophora*, with average frequency ranging from 2.0 to 23.2 (Table 2).

Free-living nematodes were found in all the soil samples. The highest average populations were observed in the municipalities of Umuarama, Alto Piquiri and Tapejara, with 502, 487 and 412 specimens, respectively, while the lowest populations were found in São Tomé, Cianorte and Tapira (97, 69 and 48 specimens, respectively).

DISCUSSION

The high diversity of nematode genera associated with sugarcane found in this study is in line with the results of Showler *et al.* (1990) and Bond *et al.* (2000), in the state of Louisiana, USA, where the genera observed were *Meloidogyne*, *Pratylenchus*, *Trichodorus*, *Paratrichodorus*, *Mesocriconema*, *Helicotylenchus* and *Tylenchorhynchus*. Bond *et al.* (2000) working in areas with three to five ratoons, found highest frequencies for *Tylenchorhynchus*, *Mesocriconema* and *Pratylenchus*, present in 93.5%, 88.2% and 79.5% of samples, respectively. *Meloidogyne*, which in this study occurred in 96.8% of root samples and 66.5% of soil samples, was observed in just 8.6% of samples in the Louisiana studies. Authors observed that with the exception of *Pratylenchus*, the major nematode numbers were associated with the sandy soils which are characteristic of the target region.

Bridge *et al.* (1996) analyzed samples of sugarcane roots and soil collected from six different areas in the district of Corozal, Belize. Nine species of phytonematodes were found: *Criconemella sphaerocephala*, *Helicotylenchus* sp., *H. microlobus*, *H. microcephalus*, *Meloidogyne* sp., *Peltamigratus christiei*, *Pratylenchus* sp., *Rotylenchulus reniformis* and *Tylenchorhynchus* sp.

In Brazil, a great diversity of nematodes associated with sugarcane rhizosphere has also been identified in prior surveys. Similar genera to those observed in this study were verified by Novaretti *et al.* (1974) in the state of São Paulo. In the states of Alagoas and Sergipe, high numbers of the genera *Pratylenchus*, *Helicotylenchus* and *Mesocriconema* were observed by Cruz *et al.* (1986).

Among the factors that may contribute towards the variation in nematode species and the numbers observed are the soil type, the variety of sugarcane and climatic conditions in the sampled region (Novaretti and Nelli, 1980; Moura *et al.*, 1999; Bond *et al.*, 2000). Another contributing factor towards the variation in populations is plant age, as observed by Hall and Irey (1990) in Florida, where the smallest populations were observed in young sugarcane. The greater number of nematodes observed in this study may be conferred on the plant age, ranging from one to eight years, and on the history of sugarcane crop in sample areas.

Previous studies have shown that nematodes of the genus *Pratylenchus* tend to be more common in clay soils (Hall and Irey, 1990; Bond *et al.*, 2000). However, the samples obtained in this study came from the Arenito Caiuá region (Paraná, Brazil), where soils of sandy and sandy-loam textures predominate. Even so, high populations of *Pratylenchus* were observed, with average frequency ranging between 85.3% and 94.3% in root and soil samples, respectively.

As was observed in this work, high population densities of *Meloidogyne* and *Pratylenchus* have been consistently recorded in Brazil. Novaretti *et al.* (1990) described these genera as the principal nematodes present in sugarcane in Piracicaba, São Paulo. In Pernambuco, high frequencies of *Meloidogyne* and *Pratylenchus* were observed in sugarcane crop areas, with *Meloidogyne* the main nematode related to variable crop losses, particularly when associated with *Pratylenchus* and *Helicotylenchus* (Moura *et al.*, 1999). Later, Moura *et al.* (2000) reported 11 different genera of nematodes associated with sugarcane crop areas in the Northeast region of Brazil, and once again *Meloidogyne* and *Pratylenchus* were the predominant genera.

In the root samples collected for this study, the numbers of free-living nematodes and the genus *Helicotylenchus* were low, when they were present. This is consistent with data obtained by others, wherein the highest populations in root samples were of endoparasites (Moura and Almeida, 1981; Moura *et al.*, 1999).

In terms of species, the predominant populations found in northwestern Paraná are similar to those observed in other regions of the country. *Pratylenchus zaeae* was found in the majority of samples, followed in lower percentages by *P. brachyurus*, and the morphometric data obtained here is in line with those presented by Frederick and Tarjan (1989), Nickle (1991) and Troccoli *et al.*, (1996). In the case of root-knot nematodes, *M. javanica* was found to be the predominant species, observed in 46% of the samples analyzed, while *M. incognita* was present in 22% of the samples (data published previously by Severino *et al.*, 2008). In the work conducted by Gomes and Novaretti (1985) in the Ribeirão Preto region of São Paulo state, within the genus *Pratylenchus*, 85% belonged to *P. zaeae*, and among *Meloidogyne*, 83% were accounted

for by *M. javanica*; the latter was cited as the most widespread root-knot nematode in the sugarcane crop areas (Dinardo-Miranda *et al.*, 2003).

The species *H. dihystra* was the only one found within the genus, and the morphometry was in line with that presented by Fortuner (1984) and Wouts and Yeates (1994). In total, 35 species of *Helicotylenchus* found in sugarcane throughout the world have been described (Cadet and Spaul, 2005). Nevertheless, there is a lack of research on *Helicotylenchus* species in this crop. In Brazil, *Helicotylenchus* species have frequently been reported in studies conducted in the country's Northeast region (Chaves *et al.*, 2003; Rosa *et al.*, 2004).

From the present study, one can conclude that, despite variations in populations of nematodes associated with sugarcane roots and soil, *Meloidogyne* and *Pratylenchus* were the most frequently found, followed by the genus *Helicotylenchus*. Within the *Pratylenchus* and *Helicotylenchus* genera, the species *P. zaeae* and *H. dihystra* were predominant. These results may assist in nematode management, particularly when tolerant varieties are used, given that the behavior of these plants may vary according to nematode populations, crop age, soil types, and other factors.

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