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Survey of nematodes associated with sugarcane in the state of Paraná, Brazil

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ABSTRACT

Sugarcane-associated nematodes (*Saccharum* spp.) can reduce productivity up to 50%. Through the survey, it was possible to identify the main nematodes that occur in a certain region as a tool for designing the best management and control strategies. The aim of this study was to characterize the population of nematodes associated with sugarcane in the North Central, North Pioneiro and Northwest mesoregion of the state of Paraná, Brazil, quantify the nematode genera associated with the crop and identify the species of *Pratylenchus* and *Meloidogyne*. A total amount of 89 soil and root composite samples were collected in nine municipalities. Nematodes were extracted and counted in a Peters counting chamber under an optical light microscope. Morphological description followed identification keys. *Pratylenchus* spp. were identified by morphological characteristics; *Meloidogyne* spp. were identified by morphological characteristics and isoenzyme electrophoresis. Twelve genera of nematodes associated with sugarcane were identified: *Pratylenchus, Meloidogyne, Helicotylenchus, Xiphinema, Mesocriconema, Trichodorus, Aphelenchus, Hoplolaimus, Tylenchus, Tylenchorhynchus, Ditylenchus*, and *Paratrichodorus*. The genera *Pratylenchus* and *Meloidogyne* were found with the highest frequencies in the roots. Among the species of *Pratylenchus*, *P. zeae* and *P. brachyurus* were found, with *P. zeae* being the most frequent. Among the *Meloidogyne* species, only *M. javanica* was found. These results are essential to aid decision making in the management of phytonematodes, mainly in the development of new control strategies and in directing genetic breeding programs for development of sugarcane cultivars for the Paraná state.

Keywords: *Saccharum* spp.; *Pratylenchus*; *Meloidogyne*; identification, nematode.

INTRODUCTION

Sugarcane (*Saccharum* spp.) stands out in the Brazilian economy, due to favorable soil and climate conditions for cultivation (SILVA et al., 2016) combined with high demand for sugar, ethanol and other products and by-products (KOHLHEPP, 2010). As the largest producer in the world, Brazil harvested 654.5 million tons in the 2020/21 harvest season (CONAB, 2021).

Sugarcane production can be compromised by biotic factors such as the occurrence of diseases caused by nematodes (DINARDO-MIRANDA, et al., 2010; VAN DEN HOOGEN et al., 2019). In Brazil, due to high nematode population levels associated with highly susceptible cultivars and favorable environmental conditions, it is estimated that the reduction in productivity can reach 50% (DINARDO-MIRANDA; FRACASSO, 2010).

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Meloidogyne is the most important phytonematode genera in the world (JONES et al., 2013; CARNEIRO, et al. 2020). They are known as root-knot nematodes because they induce the formation of abnormal cells in plant roots (DINARDO-MIRANDA et al., 2010; JONES et al., 2013; FERRAZ, 2018). In sugarcane, the galls are apical and of reduced size, therefore, difficult to be detected (CROW, 2004). The genus *Pratylenchus*, known as root lesion nematodes, is also important for sugarcane (JONES et al., 2013). Due to the mechanical damage caused by these phytopathogens in the plant roots, infections caused by fungi and opportunistic bacteria that cause typical necrotic lesions can occur in these organs (DINARDO-MIRANDA et al., 2010).

Surveys of nematodes that occur in sugarcane in Brazil, carried out in Rio Grande do Sul (BELLÉ et al., 2014), Pernambuco (CARDOSO et al., 2015; MARANHÃO et al., 2018), Alagoas (NORONHA et al., 2017) and São Paulo (DINARDO-MIRANDA, 2005), detected the predominance of the species *Meloidogyne javanica*, *Meloidogyne incognita*, and *Pratylenchus zeae*, which are considered the key species for sugarcane in Brazil (CADET; SPAULL, 2005). The presence of nematodes belonging to the following genera was also detected: *Helicotylenchus, Xiphinema, Hoplolaimus, Trichodorus, Paratrichodorus, Mesocriconema, Aphelenchus, Aphelenchoides*, and *Hemicycliophora* (DINARDO-MIRANDA, 2005; BELLÉ et al., 2014; CARDOSO et al., 2015; NORONHA et al., 2017; MARANHÃO et al., 2018).

In the state of Paraná, the only survey of nematodes took place between October 2005 and April 2006, concentrated in the Northwest mesoregion, in the municipalities of Cidade Gaúcha, Maria Helena, Tapira, Tapejara, Cianorte, São Tomé, Cruzeiro do Oeste, Alto Piquiri, Perobal, and Umuarama, where 74 samples with a predominance of sandy soils were collected (SEVERINO et al., 2010). After this period, the production of sugarcane in Paraná increased from 28.5 million tons cultivated in 410.9 thousand hectares in the 2005/2006 harvest season to 568.4 million tons cultivated in 8.264,4 thousand hectares in the 2020/2021 harvest season. In the Southern region, there was a small increase of 0.4% in the cultivated area; however, with a 6.6% reduction in production compared to the previous harvest. A possible explanation for this decrease in productivity is the occurrence of nematodes associated with the crop, which points to the need to update the data on nematodes occurring in sugarcane, especially in areas of the state of Paraná (CONAB, 2021). The "Rede Interuniversitária para o Desenvolvimento do Setor Sucroenergético" (RIDESA) plays an important role in the development of sugarcane cultivars with increased productivity, in addition to the search for disease-resistant cultivars. RIDESA has a partnership with several power plants concentrated. In addition to the Northwest mesoregion of the state, RIDESA has partnerships with several plants also concentrated in the Norte Pioneiro and Centro Norte mesoregions (DAROS et al., 2016), which were not covered in the previous survey. Therefore, a complementary survey to the one carried out is of great importance.

Through the survey, it is possible to identify the main nematodes that occur and, thus, help breeding programs to direct their research for resistance of cultivars to the most important and frequent nematodes, since the experiments to evaluate the resistance of sugarcane cultivars to nematodes are time-consuming and laborious. In addition, it can also direct the studies to evaluate the effectiveness of chemical nematicides as well as those of biological control agents. Therefore, the objective of this study was: (i) to identify and quantify the nematode genera associated with roots and soils in the sugarcane crop in the Northwest, North Central and North Pioneer mesoregions of the state of Paraná and (ii) to identify the species of *Pratylenchus* and *Meloidogyne* in these regions.

MATERIAL AND METHODS

Sample locations and collections

A total of 89 soil and root samples were collected from sugarcane fields belonging to power plants from RIDESA's partners, located in nine municipalities in three mesoregions of the state of Paraná: Northwest (Paranavaí and Itaúna do Sul), North Central (Florida, Jandaia do Sul, Lobato, and Santa Fé) and North Pioneiro (São Sebastião da Amoreira, Nova América da Colina, and Jacarezinho), during the months of January, February and March of 2019 and 2020 (Fig. 1). The soils of the sampled mesoregions were sandy and clay.

Sugarcane cultivars 'RB966928', 'RB867515' and 'RB855453' were the most planted in the sampled areas, and represented 21.7, 9.6 and 8.4% of the samples, respectively. The other cultivars sampled were 'RB928064', 'RB835054', 'RB036066', 'RB966328', 'CV7231', 'CTC4', 'CTC9001', 'CTC12', 'IACSP955000'. The number of cuttings of the ratoons ranged from 2 to 12 years, and the samples were predominantly from 8-year-old cane fields.

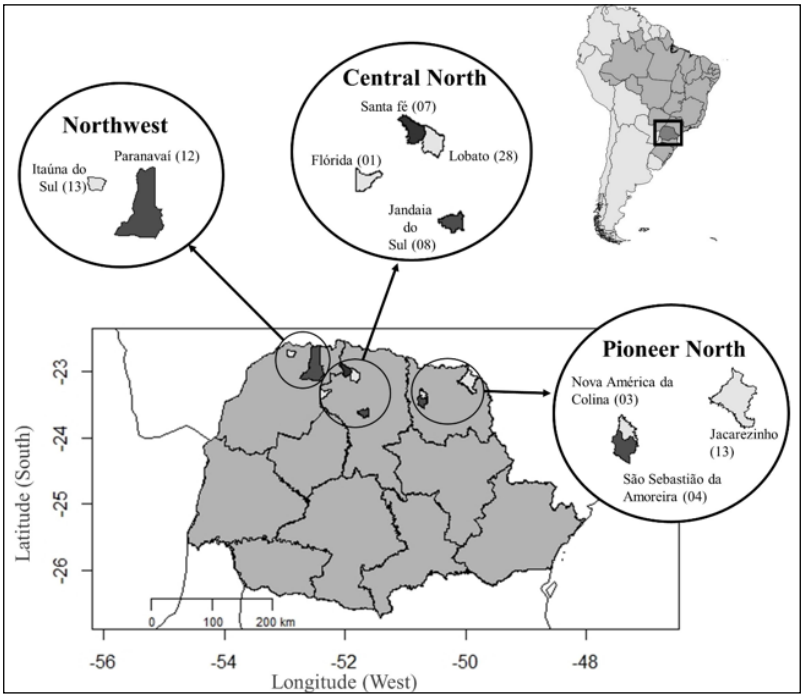

Figure 1. Regions and municipalities of the state of Paraná, Brazil, with emphasis on the Mesoregions, where soil and roots associated with the rhizosphere of sugarcane were collected to characterize the nematode population.
Source: Authors

Samples were collected in plots where a reduction in productivity was observed, possibly related to the presence of nematodes. Each sample consisted of 15 to 20 subsamples collected at random points (zig-zag) within a plot. The collection was carried out with the aid of a machete, hoe and shovel, collecting subsamples at a depth of 20 to 40 cm, according to the methodology proposed by NOVARETTI (2011). The subsamples were homogenized in a bucket and aliquots containing approximately 1 kg of soil and 0.1 kg of roots were collected and placed in properly identified plastic bags (NOVARETTI, 2011; MACHADO et al., 2019). The aliquots were sent to the Laboratory of Nematology of the Diagnostic Center Marcos Enrietti, located on the campus of the Agricultural Sciences Sector at Universidade Federal do Paraná (Curitiba, Paraná) for the extraction, identification and quantification of phytonematodes.

Extraction and fixation of nematodes

The roots of each sample were washed, and a 10 g aliquot of roots was weighed, crushed and floated in sucrose with kaolin, according to the methodology proposed by COOLEN; D'HERDE (1972). The extraction of nematodes from the soil was carried out using the centrifugal flotation technique in sucrose solution (JENKINS, 1964), from a volume of 100 cm^3 of each soil sample. The suspension containing the nematodes obtained from the extractions was reduced to 4 mL with the aid of a syringe. The concentrated sample was subjected to heating at a temperature of 55–65 °C in a water bath for 1 min. A total of 4 mL of 5% formalin was added to the suspensions and, after 1 h, the suspension was reduced again to 4 mL (HOOPER, 1986).

Morphological identification of genera and quantification

Semipermanent slides were prepared with about 20 individuals from the suspension obtained from each sample (TIHOHOD, 1993). The slides were observed under an optical microscope to identify the nematode genera. Nematodes were photographed using spot advanced software to help their identification. The identification of the nematode genera present in each sample was based on morphological characteristics with the aid of dichotomous keys (JAIRAJPURI; AHMAD, 1992; MAI et al., 1996; HUNT; HANDOO, 2009; FERRAZ, 2016). The quantification of individuals of each genus was performed in a Peters counting chamber. The suspensions of samples in which *Meloidogyne* juveniles were observed were deposited in autoclaved soil (1 h at 121 °C) with 'Santa Clara' tomato plants (*Solanum lycopersicum* L.), a cultivar susceptible to *Meloidogyne*. The plants were kept in a greenhouse (± 25 °C) to obtain sexually mature females.

Data were analyzed to characterize the populations and determine the mean population density (MPD), that is, the mean number of individuals per 10 g of root, and per 100 cm^3 of soil samples. The frequency of occurrence was calculated through the number of samples with the presence of the genus divided by the total number of samples from the municipality or region × 100.

Identification of *Pratylenchus* and *Meloidogyne* species

Of the 89 samples, 22 samples were selected (4 from the Northwest mesoregion, 4 from the North Central mesoregion, and 14 from the North Pioneiro mesoregion) for identification of *Pratylenchus* to species level. The largest number of samples was concentrated in the North Pioneiro mesoregion because of the predominance of clay soils, thus complementing the survey carried out by SEVERINO et al. (2010), who sampled only sandy soils (Northwest mesoregion). From the suspension obtained from each sample, individuals of *Pratylenchus* spp. were retrieved and semipermanent slides were prepared (TIHOHOD, 1993). The species of *Pratylenchus* were identified by the following morphological characteristics: verification of spermatheca shape and if functional, that is, if it had sperm; body width; number of rings in the labial region; shape of the nodules and length of the stylet; vulva position; tail shape; and distance from the labial region to the vulva in percentage of total body length (V%) (CASTILLO; VOVLAS, 2007).

The soil and roots remaining from the samples containing *Meloidogyne* juveniles were placed in 2 L pots, totaling 62 samples (26 from the Northwest mesoregion and 36 from the North Central mesoregion). Tomato plants were grown in the pots and, after four months, were evaluated for the presence of galls. The roots that showed galls were separated. Females were extracted directly from the root of tomato plants and species of the genus *Meloidogyne* were identified by the morphology of their perineal pattern. Ten sections of the perineal region per sample were placed on a microscope slide with a drop of glycerin, then the perineal pattern of aberrant females was compared and classified according to specific characteristics of each species (EISENBACK et al., 1980).

To confirm the morphological identification of *Meloidogyne* species, the samples were biochemically characterized by electrophoretic patterns of esterase isoenzymes (CARNEIRO et al., 2000; ESBENSHADE; TRIANTAPHYLLOU 1985, 1990). Twenty mature females from each sample were extracted directly from the root and deposited in polypropylene microtubes with 20 µL of extraction solution (2 mL of glycerol, 0.2 g of Triton X-100, and 7.8 mL of distilled water) and kept on ice. The females were macerated and placed in 8% polyacrylamide gel cavities with a 10 µL Hamilton syringe (ALFENAS, 2006). Females of *M. javanica* from a pure population were used as a standard for band comparison. The polyacrylamide gel was placed in a tub with a buffer, connected to a 90 V source and kept in a refrigerator (6 ± 2 °C). Two wells received 0.1% bromophenol blue dye to make the migration process of the samples in the gel visible. When the dye reached 6 cm from the wells (after approximately three and a half hours) the source was turned off (CARNEIRO et al., 2000). Subsequently, the gel was deposited in a phosphate buffer solution (50 mg of Fast Blue RR Salt and 1.5 mL of α-naphthyl acetate 1%) to make the bands permanently visible, and then placed in an oven at 37 °C for 20 min. The gel was fixed in a solution containing 10% acetic acid and 40% methyl alcohol, for 30 min. After fixation and drying in an oven, the gel was cut, photographed and then placed between two layers of cellophane for storage.

RESULTS

Morphological identification and quantification of genera

The evaluation of nematodes in sugarcane producing regions indicated the presence of 12 genera of nematodes in association with sugarcane, in a total of 89 soil and root samples. In all samples evaluated, more than one genus of plant parasitic nematode was detected. In the roots, the highest population densities (average number of nematodes in 10 g of roots) were for the genera *Pratylenchus* (797.88 nematodes), *Meloidogyne* (15.02 nematodes) and *Helicotylenchus* (27.5 nematodes), with frequency of occurrence of these genera in the state of Paraná of 98.8, 70.0 and 60.0%, respectively. *Pratylenchus* and *Meloidogyne* occurred concomitantly in 62.3% of the root samples. The genera *Xiphinema* (0.47 nematode), *Mesocriconema* (0.5 nematode), *Trichodorus* (0.81 nematode), and *Aphelenchus* (0.75 nematode) were also found in smaller proportions. The nematode genera with frequencies close to zero are ectoparasites and were included in the category "Others," namely *Hoplolaimus*, *Tylenchus* and *Tylenchorhynchus* (Table 1).

Table 1. Mean population density and frequency of occurrence of nematodes in sugarcane roots in the state of Paraná.

Mesoregions/ Municipalities	*Praty.*[1]		*Meloi.*[2]		*Helic.*[3]		*Xiphi.*[4]		*Mesoc.*[5]		*Trich.*[6]		*Aphe.*[7]		*Others*[8]	
	MPD[9]	Fr.[10]	MPD	Fr.	MPD	Fr.	MPD	Fr.	MPD	Fr.	MPD	Fr.	MPD	Fr.	MPD	Fr.
Northwest (25)[11]	2273.7	100.0	17.6	75.0	7.5	44.0	-	-	0.9	20.0	0.5	12.0	0.2	4.0	-	-
Paranavaí (12)	86.0	100.0	11.2	83.3	15.6	91.6	-	-	0.4	16.6	-	-	0.4	8.3	-	-
Itaúna do Sul (13)	4293.0	100.0	16.7	38.4	-	-	-	-	1.5	23.0	-	-	-	-	-	-
North Central (44)	332.2	97.7	14.3	59.0	8.2	54.0	-	-	0.5	2.2	0.6	2.2	1.5	4.5	1.9	9.0
Lobato (28)	369.0	100.0	6.3	57.0	13.2	85.0	-	-	0.0	3.5	10.9	78.0	-	-	2.9	14.0
Santa Fé (7)	209.2	100.0	52.1	71.0	10.0	100.0	-	-	-	-	-	-	-	-	-	-
Flórida (1)	1595.0	100.0	10.0	100.0	-	-	-	-	-	-	-	-	-	-	-	-
Jandaia do Sul (8)	158.0	87.5	9.6	50.0	24.3	37.0	-	-	2.8	12.0	3.7	12.0	2.8	12.5	-	-
North Pioneiro (20)	39.0	100.0	17.7	80.0	19.0	95.0	2.1	20.0	0.1	5.0	6.9	44.0	3.1	30.0	3.5	11.0
Jacarezinho (13)	18.6	100.0	26	92.0	23.0	100.0	2.6	15.0	-	-	0.7	15.0	3.7	61.5	3.7	61.5
Nova América da Colina (3)	34.1	100.0	-	-	7.5	66.0	-	-	0.8	33.0	-	-	10.8	100.0	-	-
São Sebastião da Amoreira (4)	108.7	100.0	3.7	100.0	14.3	100.0	1.8	50.0	-	-	0.6	25.0	7.5	75.0	7.5	25
Paraná (89)[12]	797.8	98.8	15	70.0	27.5	60.0	0.4	2.7	0.5	8.9	0.8	7.8	0.75	7.8	1.17	17.0

[1]*Pratylenchus* spp.; [2]*Meloidogyne* spp.; [3]*Helicotylenchus* spp.; [4]*Xiphinema* spp.; [5]*Mesocriconema* spp.; [6]*Trichodorus* spp.; [7]*Aphelenchus* spp.; [8]Others: *Hoplolaimus* spp.; *Tylenchus* spp. and *Tylenchorhynchus* spp.; [9]MPD: Mean population density (individuals × 10 g^{-1} of root); [10]Fr.: Frequency of occurrence (%); [11]Number of root samples analyzed in the mesoregion of the state of Paraná; [12]Total of root samples analyzed in the state of Paraná.

In the larger soil samples, the population densities (mean number of nematodes in 100 cm^3 of soil) were *Pratylenchus* (131.4 nematodes), *Meloidogyne* (7.5 nematodes), and *Helicotylenchus* (62.1 nematodes), and the frequency of occurrence of these genera in the state of Paraná were 86.5, 32.5, and 88.7%, respectively. The genera *Mesocriconema* (9.47 nematodes), *Xiphinema* (5.27 nematodes), *Trichodorus* (8.28 nematodes), and *Aphelenchus* (0.36 nematode) were also found with lower frequencies. The genera *Hoplolaimus, Tylenchus, Tylenchorhynchus, Ditylenchus*, and *Paratrichodorus* presented frequencies close to zero, and were grouped in the category "Others." The genera *Pratylenchus* and *Meloidogyne* occurred concomitantly in 32.5% of the soil samples from the state of Paraná (Table 2).

Table 2. Average population density and frequency of occurrence of nematodes in sugarcane soil in the state of Paraná.

Mesoregions/ Municipalities	*Praty.*[1]		*Meloi.*[2]		*Helic.*[3]		*Xiphi.*[4]		*Mesoc.*[5]		*Trich.*[6]		*Aphe.*[7]		*Others*[8]	
	MPD[9]	Fr.[10]	MPD	Fr.	MPD	Fr.	MPD	Fr.	MPD	Fr.	MPD	Fr.	MPD	Fr.	MPD	Fr.
Northwest (25)[11]	286.9	84	7.2	36.0	8.5	68	0.9	16	7.2	40.0	-	-	-	-		
Paranavaí (12)	70.0	66.6	0.8	33.3	11.0	75.0	-	-	0.2	8.3	-	-	-	-	-	-
Itaúna do Sul (13)	487.1	100.0	13.0	38.4	5.5	61.5	17.5	30.7	52.1	69.2	2.3	23.0	-	-		-
North Central (44)	85.6	93.0	5.1	36.3.0	50.3	88.6	1.9	18.0	2.6	31.8	5.3	25.0	0.5	2.3	2.6	25.0
Lobato (28)	102.0	96.0	3.0	35.7	37.9	85.7	12.2	85.7	2.4	21.4	10.9	78.5	-	-	3.7	35.7
Santa Fé (7)	58.9	100.0	4.2	57.0	51.7	100.0	10.0	71.4	0.2	42.8	19.2	85.7	-	-	-	-
Flórida (1)	90.0	100.0	5.0	100.0	22.5	100.0	7.5	100.0	2.5	100.0	10.0	100.0	-	-	-	-
Jandaia do Sul (8)	50.9	75.0	13.4	25.0	96.2	87.5	1.2	25.0	5.0	50.0	11.5	50.0	0.6	25.0	1.6	12.5
North Pioneiro (20)	37.7	100.0	13.1	55.0	165.0	100.0	22.5	100.0	2.2	25.0	6.2	45.0	1.3	20.0	2.5	35.0
Jacarezinho (13)	24.0	100.0	18.8	61.5	196.3	100.0	31.9	100.0	0.1	7.6	9.6	69.2	-	-	0.8	23.0
Nova América da Colina (3)	40.0	100.0	4.1	33.3	59.1	100.0	5.0	100.0	9.1	66.6	-	-	1.7	33.3	3.3	50.0
São Sebastião da Amoreira (4)	80.6	100.0	1.2	50.0	146.0	100.0	5.0	20.0	3.7	50.0	11.8	50.0	5.0	75.0	7.5	50.0
Paraná (89)[12]	131.4	86.5	7.5	32.5	62.1	88.7	5.8	60.6	9.4	28.0	8.2	52.0	0.4	6.7	1.9	20.2

[1]*Pratylenchus* spp.; [2]*Meloidogyne* spp.; [3]*Helicotylenchus* spp.; [4]*Xiphinema* spp.; [5]*Mesocriconema* spp.; [6]*Trichodorus* spp.; [7]*Aphelenchus* spp.; [8]Others: *Hoplolaimus* spp.; *Tylenchus* spp.; *Tylenchorhynchus* spp.; *Ditylenchus* spp., and *Paratrichodorus* spp. [9]MPD: Mean population density (individuals × 100 cm^3 of soil); [10]Fr.: Frequency of occurrence (%); [11]Number of soil samples analyzed in the mesoregion of the state of Paraná; [12]Total of soil samples analyzed in the state of Paraná.

Identification of *Pratylenchus* and *Meloidogyne* species

From 22 samples selected for morphological identification of *Pratylenchus* species, *P. zeae* and *P. brachyurus* were identified. In the roots, the frequencies of occurrence were 95.5 and 77.27%, and the MPD were 57.3 and 22.21 individuals for *P. zeae* and *P. brachyurus*, respectively. In the soil, the frequencies of occurrence were 86.4 and 54.5% with MPD of 30.9 and 12.1 individuals for *P. zeae* and *P. brachyurus*, respectively. *Pratylenchus brachyurus* nematode was not detected only in Paranavaí municipality (Table 3). Individuals of the species *P. zeae* had flattened basal nodules, labial region continuous with the body contour, vulva located close to the central region of the body and a subacute tail. Individuals of the species *P. brachyurus* had spherical basal nodules, robust stylet, position of the vulva near the end of the body, hemispherical tail with a smooth terminus and an angular labial region showing two rings and narrower than the body diameter (CAFÉ FILHO; HUANG, 1989; CASTILLO; VOVLAS, 2007).

Table 3. Mean population density and frequencies of Pratylenchus species found in sugarcane soil and root in the state of Paraná.

Mesoregions/ Municipalities	*Pratylenchus zeae*				*Pratylenchus brachyurus*			
	Soil (100 cm^3)		Root (10 g)		Soil (100 cm^3)		Root (10 g)	
	MPD[1]	Fr.[2]	MPD	Fr.	MPD	Fr.	MPD	Fr.
Northwest (4)[3]	3.7	50.0	108.7	100.0	-	-	51.8	100.0
Paranavaí (4)	3.7	50.0	108.7	100.0	-	51.8	100.0	3.7
North Central (4)	21.6	75.0	59.3	100.0	17.5	25.0	31.2	50.0
Jandaia do Sul (4)	21.6	75.0	59.3	100.0	17.5	25.0	31.2	50.0
Norte Pioneiro (14)	36.7	100.0	40.7	92.8	11.6	78.6	9.7	78.5
Jacarezinho (7)	14.6	100.0	15.0	86.0	10.0	71.0	5.0	57.0
Nova América da Colina (3)	30.8	100.0	25.8	100.0	9.2	100.0	8.3	100.0
São Sebastião da Amoreira (4)	80.0	100.0	90.6	100.0	16.7	75.0	15.6	100.0
Paraná (22)[4]	30.9	86.4	57.3	95.5	12.1	54.5	22.2	77.2

[1]MPD: Mean population density.; [2]Fr.: Frequency of occurrence (%); [3]Number of samples collected in the mesoregion of the state of Paraná; [4]Total of samples analyzed in the state of Paraná.

Of the 62 samples used to identify *Meloidogyne* species, only 12 samples presented galls. The samples were from the Northwest mesoregion (9 samples from the municipality of Paranavaí and 3 from the municipality of Itaúna do Sul). The perineal sections of the females of the 12 samples showed a marked lateral line dividing the perineal with the configuration of typical streaks for the species *M. javanica* (EISENBACK et al., 1980). The 12 samples morphologically identified as *M. javanica* were confirmed by isoenzyme electrophoresis in the J3 phenotype pattern, showing three bands with a migration ratio in: 1.00, 1.07 and 1.17, typical for the *M. javanica* species (CARNEIRO et al., 2000), indicating that there were no mixed populations of *Meloidogyne* species.

DISCUSSION

The population diversity of phytonematodes associated with sugarcane cultivations in the state of Paraná was evidenced in this study through the identification of twelve genera of phytonematodes. The following genera were detected: *Pratylenchus, Meloidogyne, Helicotylenchus, Xiphinema, Mesocriconema, Trichodorus, Aphelenchus, Hoplolaimus, Tylenchus, Tylenchorhynchus, Ditylenchus*, and *Paratrichodorus*. The genera *Pratylenchus* and *Meloidogyne*, considered the most important for sugarcane, were found in higher densities in the roots. Among the species of *Pratylenchus*, the species *P. zeae* and *P. brachyurus* were found, being the most frequent. Among the species of the genus *Meloidogyne*, only the species *M. javanica* was found. These results are essential to guide genetic improvement programs for sugarcane resistant to phytonematodes of importance in the state of Paraná cultivations.

The first and, until then, only survey of sugarcane in Paraná was carried out from October 2005 to April 2006 and was restricted to the Northwest mesoregion, where *Pratylenchus*, *Meloidogyne*, and *Helicotylenchus* were found in the frequencies of occurrence 96.8, 85.3 and 82.9%, respectively (SEVERINO et al., 2010). In this new survey, three mesoregions were evaluated, being more comprehensive, including, in addition to the Northwest region, the North Central and North Pioneiro regions. The

results of this study showed that the frequency of occurrence of the genus *Pratylenchus* was similar in both surveys. However, *Meloidogyne* had a lower frequency of occurrence in soil and roots in the survey carried out in this study. The difference between the frequency of occurrences may be associated with environmental factors, temperature, water content in the soil, sugarcane cultivar and management adopted by producers, as they interfere in the density and frequency of genera associated with the sugarcane rhizosphere (NOVARETTI; NELLI, 1980; HUANG; PEREIRA, 1994; MOURA et al., 1999; BOND et al., 2000; CARDOSO et al., 2015, 2016). The age of the ratoon is also directly proportional to the population density of nematodes, with a tendency towards population increase in ratoon crops from one to eight years old, compromising the sugarcane field longevity (CADET; BOER, 1990; HALL; IREY, 1990; BLAIR; STIRLING, 2007; SEVERINO et al., 2008).

The results of this study point to the widespread occurrence of *Pratylenchus* in samples collected from soil and root, and the municipalities in the Northwest mesoregion had the highest population densities. These results observed in the Northwest mesoregion are possibly associated with sandy soils that are predominant and that favor the nematodes of the genus *Pratylenchus* (FERRAZ; BROWN, 2016). It is important to highlight that the lesion nematodes are distributed in almost all regions where there is sugarcane cultivation, being the most important genus associated with the culture (CADET; SPAULL, 2005). In the present study, the presence of a mixed population of two *Pratylenchus* species was identified, with *P. zeae* having a higher mean density than *P. brachyurus*, corroborating other surveys of nematodes in sugarcane in Brazil (DINARDO-MIRANDA et al., 2004, 2019; OLIVEIRA et al., 2008; CHAVES et al., 2009; SEVERINO et al., 2010). Likewise, in surveys in sugarcane plantations in India and South Africa, *P. zeae* has been the most found species of the genus Pratylenchus (CADET; SPAULL, 2005; BERRY et al., 2007). However, the occurrence of *P. brachyurus* has become frequent and worrying producers and researchers in the sector. BARBOSA et al. (2013) revealed that *P. brachyurus* was more aggressive than *P. zeae*, causing greater damage to the development of sugarcane plants.

When considering the economic damage level of *Pratylenchus* nematodes in sugarcane, NOVARETTI (1997) indicates that 50 to 80 individuals of *Pratylenchus* per gram of roots have been considered as medium and high population densities that result in losses (DINARDO-MIRANDA; FERRAZ, 1991; NOVARETTI, 1995; DINARDO-MIRANDA et al., 1998; MOURA et al., 1999). Based on this criterion, the population levels of *Pratylenchus* in this study may be causing losses in sugarcane production in the state of Paraná, as the MPD of *Pratylenchus* in the state reached 79.7 per gram of root (Table 1). In the municipalities of Itaúna do Sul and Flórida, the population is much above the damage level, with an average density of 429.3 and 159.5 nematodes per gram of root, respectively (Table 1). Considering that population densities of 54 individuals of *P. zeae* per gram of root were responsible for a 35% reduction in sugarcane production (DINARDO-MIRANDA; FERRAZ, 1991), management and control strategies must be applied to mitigate possible damage and losses in production.

Meloidogyne javanica was the only species of the root-knot nematode identified in this survey. The presence of this species was more observed in the roots. These results were expected from sedentary endoparasite pathogens, corroborating previous study in which *M. javanica* were the predominant species in sugarcane roots (SEVERINO et al., 2008). Other species of root-knot nematodes such as *M. paranaensis* reported in Paraná (SEVERINO et al., 2010) and *M. ethiopica* in Rio Grande do Sul (BELLÉ et al., 2017) may represent a problem for the future of sugarcane production. However, no *M. paranaensis* or *M. ethiopica* were found in the present survey.

The estimation of damage levels caused by *Meloidogyne* is complex; therefore, it is little studied in sugarcane, due to several abiotic factors that interfere in the analysis and may or may not be related to parasitism (CHAVES et al., 2009). However, NOVARETTI (1997) pointed out that eight individuals per gram of roots indicate high population density. In this study, the *Meloidogyne* density did not reach this average.

Nematodes of the genus *Helicotylenchus* were the third most common genus found in roots, with 60.6% frequency and population density of 27.5 nematodes (Table 1). In the soil, it was the second most found with 88.7% of frequency in the samples and population density of 62.1 nematodes (Table 1). *Helicotylenchus* is a very common ectoparasite found in sugarcane surveys in Brazil, mainly in the northeast region (CHAVES et al., 2003; SEVERINO et al., 2010) and in predominantly sandy soils (RIMÉ et al., 2003; SHOKO; ZHOU, 2009; WINARTO et al., 2019). A total of 35 *Helicotylenchus* species have been reported in association with sugarcane (CADET; SPAULL, 2005). However, *H. dihystera* is the most common in sugarcane fields and when associated with other ectoparasites it is related to crop damage (RIMÉ et al., 2003). RAO; SWARUP (1974) indicate that two individuals of *H. dihystera* per gram of soil in sugarcane is the minimum to cause losses in it. In the present study, only the municipality of Jacarezinho came close to this level of damage, with an MPD of 1.96 individual per gram of soil (Table 2).

Nematodes of the *Xiphinema* genus were found in 45.4% of the soil samples. SEVERINO et al. (2010) found the genera in 54.7% of the samples in Paraná. Although in Brazil this genus is not considered economically important for sugarcane, nematodes of the genus *Xiphinema* are important viral vectors and, when associated with other ectoparasites, can reduce sugarcane productivity (CADET, SPAULL; 2005). The other genera of nematodes, although found less frequently and not

considered economically important pathogens (MARANHÃO et al., 2018), *Aphelenchus, Mesocriconema, Tylenchorhynchus, Trichodorus, Hoplolaimus, Tylenchus*, and *Ditylenchus*, when at high population levels, can severely interfere with plant development (SHOWLER et al., 1990; BOND et al., 2000).

CONCLUSIONS

Twelve genera of nematodes associated with sugarcane were identified: *Pratylenchus, Meloidogyne, Helicotylenchus, Xiphinema, Mesocriconema, Trichodorus, Aphelenchus, Hoplolaimus, Tylenchus, Tylenchorhynchus, Ditylenchus*, and *Paratrichodorus*. The genera *Pratylenchus* and *Meloidogyne* were found in higher frequencies in the roots. *Pratylenchus zeae* was more frequent than *P. brachyurus* among *Pratylenchus* species, and *M. javanica* was the only species of *Meloidogyne*.

AUTHORS' CONTRIBUTIONS

Conceptualization: Duarte, H.S.S.; Ruaro, L.; Oliveira, R.A.; Martinha, D.D. **Data curation:** Martinha, D.D.; Silva, M.C.C.; Maceda, A.; Hahn, M.H. **Formal analysis:** Martinha, D.D.; Candido, M.C. **Funding acquisition:** Oliveira, R.A. **Investigation:** Martinha, D.D. **Methodology:** Maceda, A.; Ruaro, L.; Duarte, H.S.S.; Hahn, M.H. **Project administration:** Duarte, H.S.S. **Resources:** Oliveira, R.A. **Supervision:** Duarte, H.S.S. **Validation:** Duarte, H.S.S.; Calegario, R.F.; Hahn, M.H.; Ruaro, L. **Visualization:** Martinha, D.D.; Silva, M.C.C.; Maceda, A.; Hahn, M.H.; Calegario, R.; Ruaro, L.; Oliveira, R.A.; Duarte, H.S.S. **Writing – original draft:** Martinha, D.D. **Writing – review & editing:** Calegario, R.; Ruaro, L.; Oliveira, R.A.; Duarte, H.S.S.

AVAILABILITY OF DATA AND MATERIAL

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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CONFLICTS OF INTEREST

The authors certify that they have no commercial or associative interest that represents a conflict of interest in connection with the manuscript.

ETHICAL APPROVAL

Not applicable.

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