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CROP SCIENCE

Impact of different *Meloidogyne* species on the development of sugarcane plants

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Abstract: This study aimed to evaluate the impact of *Meloidogyne javanica*, *Meloidogyne incognita*, and *Meloidogyne arenaria* on different aspects of the development of sugarcane plants under greenhouse conditions. For this purpose, seedlings of the RB867515 genotype were individually inoculated with 5,000 eggs + second-stage juveniles of their respective nematodes/plant, and non-inoculated plants were used as control. After 330 days of inoculation, the plants were removed from the pots, and the following characteristics were evaluated: fresh mass of the aerial part and root system; leaf area; leaf chlorophyll index; culm diameter; fresh mass of culms; broth volume; contents of neutral and acid detergent fiber, cellulose, hemicellulose, lignin, apparent sucrose in broth, and reducing sugars in broth; total soluble solids concentration. Subsequently, the final population of nematodes in the root system of inoculated plants was determined to calculate the reproduction factor of nematodes. The results showed that all tested *Meloidogyne* species negatively affected plant development and the composition of some analyzed fractions, in comparison to the non-inoculated control. However, the presence of the root-knot nematode in sugarcane plants increased the contents of neutral and acid detergent fiber, cellulose, hemicellulose, lignin, and reducing sugars, regardless of the *Meloidogyne* species.

Key words: *Saccharum* spp, RB86751, root-knot nematode, plant development.

INTRODUCTION

Sugarcane (*Saccharum* spp. L hybrid) is one of the most important crops in the global socio-economic scenario, including Brazil. World sugarcane production is approximately 2.5 billion tons per year. In Brazil, its production is around 658 million tons in more than 9.5 million cultivated hectares. Moreover, this country is responsible for 25% of the global production with the raw material destined to the production of sugar, alcohol, sugarcane liquor, and also for animal feed (CONAB 2019).

Despite the growing increase in the area and productivity of sugarcane in recent harvests, several factors have been limiting production, including the presence of plant-parasitic nematodes. Different species of these pathogens negatively affect crops in practically all every region of the world where sugarcane is grown. More than 300 species of plant-parasitic nematodes, distributed in 48 genera, are associated with sugarcane crops, with an estimated average loss of 20% per year in the world (Cadet & Spaull 2005). However, the damage may vary depending on the species involved, their population levels, the susceptibility of the sugarcane variety, and the crop period of the year (Chaves et al. 2009).

In Brazil, the root-knot nematode (*Meloidogyne* spp.) and root-lesion nematode (*Pratylenchus* spp.) are the most frequent

plant-parasitic nematodes damaging sugarcane crops (Dinardo-Miranda 2008, Severino et al. 2010, Bellé et al. 2014). In the northeastern region and the São Paulo state, southeastern Brazil, the occurrence of *Meloidogyne javanica* and *Meloidogyne incognita* was verified in sugarcane crops (Dinardo-Miranda et al. 2003, Noronha et al. 2017). In the southern region, in a nematological survey conducted in the Paraná state, Severino et al. (2008) found that the most common root-knot nematode species was *M. javanica*, followed by *M. incognita*.

The importance of root-knot nematodes for sugarcane crops can be particularly verified with the decrease in productivity associated with difficulties in managing these pathogens (Barros et al. 2005, Dinardo-Miranda 2008). Several control methods aimed at decreasing plant-parasitic nematode populations in the crop, at levels below the economic damage threshold, have been studied. These include the use of nematicides, crop rotation, soil tillage, the incorporation of organic matter, and the use of sugarcane plants with resistant or tolerant genotypes. Overall, genetic resistance is considered one of the most desired control practices since it is economically viable, accessible to producers, and does not pose risks to human health and the environment. However, so far, there are no available sugarcane varieties resistant to root-knot nematodes on the Brazilian market (Dias-Arieira et al. 2010, Santos et al. 2012, Bellé et al. 2017).

Considering the relevance of root-knot nematodes for the sugarcane crop, information on the genetic resistance to such pathogens is essential for adopting joint management strategies. Thus, this study aimed to assess the impact of *M. javanica*, *M. incognita*, and *Meloidogyne arenaria* on different aspects of the development and composition of the aerial part of sugarcane.

MATERIALS AND METHODS

The experiment was conducted from December 2016 to November 2017, on screens, in the Agroenergy Sector of Embrapa Temperate Climate ("Embrapa Clima Temperado"). The RB867515 genotype was used in this study since it represents 27% of the cultivated sugarcane area in Brazil (Braga-Junior et al. 2017), whose seedlings were produced according to the production system of pre-budded seedlings, adapted from Landell et al. (2013).

Pure populations of *M. javanica* (Est J3), *M. incognita* (Est I2), and *M. arenaria* (Est A2) were used as inoculums, which were reproduced and kept in tomato plants (cv. Rutgers). These were maintained in pots with sterile soil in a greenhouse (25 ± 3 °C), and the purity of each one of them was verified, periodically, by electrophoresis with the esterase isoenzyme (Carneiro & Almeida 2001).

Individual RB867515 sugarcane plants were kept in 20L pots with sterile soil (mixture of vermiculite + soil in a 1:3 ratio) and recommended fertilization for sugarcane crop. They were inoculated separately with a suspension of 5,000 eggs + second-stage juveniles (J_2) of each *Meloidogyne* species, according to the method of Hussey & Barker (1973) modified by Bonetti & Ferraz (1981). Non-inoculated plants of the same genotype were used as a control for comparison of vegetative development. The assay followed the completely randomized design with six replicates.

After 330 days of inoculation, the aerial part of each plant was separated from their roots, and their fresh mass (aerial part and roots) was evaluated (g). The following measurements were also performed: plant height (cm); culm diameter (cm); leaf area (cm²) (Hermann & Câmara 1999); leaf chlorophyll index with a ClorofiLOG1030 (Falker Automação Agrícola, Brazil); fresh mass

of culms. In addition, the number of tillers, culms, and galls in the root system of each plant was counted.

Next, eggs + J_2 were extracted from the roots of each plant (final population), according to the methodology mentioned above, to quantify the number of nematodes per gram of roots (final population), and thus to determine their reproduction factor (RF=final population / initial population) (Oostenbrink 1966), in each replicate. The culm samples of each replicate were submitted to pressing at 250 kg/cm^2, and the broth volume was measured. Subsequently, each culm sample was triturated and dried in an oven with forced circulation (60° C for 96 hours), and then finely ground, with 50 g separated for digestibility analysis (neutral and acid detergent fiber - NDF and ADF, respectively), and contents of cellulose, hemicellulose, and lignin, through near-infrared spectroscopy (NIR) (NIR FLEX N500 model, BÜCHI, Switzerland). The contents of apparent sucrose (Pol %) and reducing sugars in broth (RS %) were also determined by NIR.

The data obtained were analyzed for normality using the Shapiro-Wilk test. The transformation [√(x+0,5)] was necessary for the number of galls variable. Subsequently, the data were submitted to analysis of variance ($p \leq 0.05$), and the averages of each treatment were compared between them by the Tukey's test at 5% probability of error, using the software GENES (Cruz 2006).

RESULTS

In this study, all tested *Meloidogyne* species reproduced and negatively affected the development and composition of some analyzed fractions of sugarcane plants when compared to non-inoculated control (Tables I - V).

Table I shows the high reproduction rates of *M. javanica*, *M. arenaria*, and *M. incognita* in sugarcane plants with the RB867515 genotype, with reproduction factor values ranging from 74 to 105. Similarly, a high number of galls was found in the roots of plants inoculated with the three species, while the density of eggs +J_2 of nematodes in the roots was higher in the treatments with *M. arenaria* and *M. incognita* compared to that observed with *M. javanica*, which was reflected in the lower reproduction factor of this same species.

There was a reduction in the fresh mass of the roots (68.6%) and aerial part (65%), number of tillers (58.3%), fresh mass of culms (50.7%), culm diameter (28.3%), and plant height (12.8%) in inoculated plants in comparison to non-inoculated plants, regardless of the *Meloidogyne* species. Likewise, for the variables, broth volume, leaf area, and leaf chlorophyll index for chlorophyll *a*, *b* and total, there were significant reductions in values (>50%) compared to non-inoculated control, regardless of the root-knot nematode species (Table III; Figure 1).

Regarding neutral detergent fiber and acid detergent fiber, there was a significant

Table I. Number of galls (NG), number of nematodes per gram of root (NNGR), and reproduction factor (RF) of Meloidogyne arenaria, Meloidogyne javanica and Meloidogyne incongita in sugarcane plants.

Species	NG		NNGR		RF	
M. arenaria	10,253	a*	684	a	98	a
M. javanica	9,687	a	396	b	74	b
M. incognita	11,494	a	751	a	105	a
CV (%)	23.51		24.19		21.16	

***Averages followed by the same letters in the column do not differ from each other by the Tukey test at 5% probability. CV: coefficient of variation.**

increase (55%) in the contents detected in inoculated plants, regardless of the nematode species, when compared to the values observed in non-inoculated plants (control) (Table IV). This increase was also observed for the contents of lignin (83.3%), cellulose (80.4%), and hemicellulose (81.3%) in the aerial part of sugarcane plants inoculated with *Meloidogyne* spp. (Table IV).

Analysis of total soluble solids in water concentration (°Brix), content of apparent sucrose in broth (Pol %), and reducing sugars in broth in sugarcane broth showed that root-knot nematodes also interfered negatively in these response variables, regardless of the *Meloidogyne* species, when compared to non-inoculated plants (control) (Table V). The average reduction in °Brix and Pol % values was 17.7% and 31.8%, respectively; however, the highest interference was observed in reducing sugars in broth values, with significant average increases of 72%.

DISCUSSION

Reproduction factor values for *M. javanica* and *M. incognita* obtained in this study for the RB867515 variety were similar to those found in other works (Chaves et al. 2009, Silva et al. 2012, Silva et al. 2016). Regis & Moura (1989) also observed RF values >27 for *M. incognita* in five varieties of sugarcane at 110 days after inoculation. It should be noted that, for *M. arenaria*, there are no studies in the literature so far focusing on its reproduction and impact on the development of sugarcane plants, although the occurrence of this species has been frequently reported in nematological surveys of sugarcane crops in

Table II. Values of fresh mass of the root system (FMRS), fresh mass of the aerial part (FMAP), plant height (PH), number of tillers (NT), number of culms (NC), and culm diameter (CD) in sugarcane plants inoculated or not with Meloidogyne spp.

Species	FMRS (g)		FMAP (g)		PH (mm)		NT		NC (plant^{-1})		CD (mm)	
M. arenaria	728.3	b*	1,358.3	b	225	b	3.4	b	3.6	b	26.1	b
M. javanica	945.0	b	1,481.7	b	226.6	b	3.6	b	3.5	b	27.2	b
M. incognita	716.7	b	1,191.7	b	217.5	b	3.3	b	3.4	b	25.6	b
Control	2,541.7	a	3,858.3	a	255.8	a	8.3	a	7.1	a	36.7	a
CV (%)	19.95		17.07		23.83		20.35		24.46		18.14	

*** Averages followed by the same letters in the column do not differ from each other by the Tukey test at 5% probability. CV: coefficient of variation.**

Table III. Values of fresh mass of culms (FMC), broth volume (BV), leaf area (cm² plant^{-1}) (LA), content of chlorophyll a (CLO a), chlorophyll b (CLO b), and total chlorophyll (CLO total) in sugarcane plants inoculated or not with Meloidogyne spp.

Species	FMC (g)		BV (L)		LA (cm² plant^{-1})		CLO a		CLO b		CLO total	
M. arenaria	918.6	b*	0.56	b	1,670.4	b	123.6	b	29.1	b	151.8	b
M. javanica	1025.5	b	0.53	b	1,740.6	b	121.6	b	28.6	b	150.3	b
M. incognita	850.5	b	0.54	b	1,743.7	b	112.1	b	34.8	b	147.0	b
Control	2725.6	a	1.5	a	3,822.7	a	272.5	a	72.6	a	345.2	a
CV %)	22.34		23.47		19.8		19.11		20.52		17.7	

*** Averages followed by the same letters in the column do not differ from each other by the Tukey test at 5% probability. CV: coefficient of variation.**

Brazil (Moura et al. 2009, Noronha et al. 2017). The lower reproduction factor values for *M. javanica* compared to *M. incognita* observed here are in line with those reported in the study of Dinardo-Miranda (1999), which showed a variation of 4.5 to 9 times higher in population levels of the first species compared to the second species.

A significant reduction in the vegetative development of sugarcane plants parasitized by *M. arenaria*, *M. javanica* and *M. incognita* resulted in a negative correlation ($p<0.05$) between the variables associated with nematodes (reproduction factor and number of galls) and those related to plants, like fresh mass of the aerial part (R=-0.80 to -0.91), root system (R=-0.72 to -0.82), and culms (R=-0.75 to -0.88); number of tillers (R=-0.67 to -0.69); plant height (R=-0.71 to -0.75); culm diameter (R=-0.70 to -0.76); leaf area (R=-0.79 to -0.91); broth volume (R=-0.81 to -0.92). Regis & Moura (1989) evaluated five genotypes of sugarcane (CB45-3, Co997, Na56-79, RB72454, RB732577) inoculated with *M. incognita*. The authors found significant reductions in the shoot and root; according to these authors, the plants were impaired by root thickening (presence of galls), necrosis, and reduction of root branches. In this context, the infection of plants with such pathogens can directly influence the number of culms and broth volume produced by plants.

The average reduction in the fresh mass of culms was 66%, regardless of the *Meloidogyne* species inoculated in sugarcane plants, corroborating the results of Dinardo-Miranda (1999). Considering the average reduction in culm production observed in this study, root-knot nematode infestation in sugarcane plantations may result in losses of about 48 t/ha per year in Brazil.

The significant reduction in the values of leaf area and leaf chlorophyll index of chlorophyll *a*, *b*, and total associated with the three species of root-knot nematodes detected in this study

Table IV. Values of neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin (Lig), cellulose (Cel), and hemicellulose (Hemicel) in sugarcane plants inoculated or not with Meloidogyne spp.

Species	ADF		NDF		Lig (%)		Cel (%)		Hemicel (%)	
M. arenaria	40.2	a*	86.4	a	5.5	a	34.7	a	45.2	a
M. javanica	39.3	a	84.5	a	5.3	a	33.9	a	44.5	a
M. incognita	42.2	a	89.0	a	5.7	a	36.4	a	47.4	a
Control	22.6	b	48.7	b	3.0	b	19.4	b	25.2	b
CV %)	21.27		21.37		15.75		21.83		22.14	

*** Averages followed by the same letters in the column do not differ from each other by the Tukey test at 5% probability. CV: coefficient of variation.**

Table V. Contents of total soluble solids in water (°Brix), content of apparent sucrose in broth (Pol %), and reducing sugars in broth (RS %) in sugarcane plants inoculated or not with Meloidogyne spp.

Species	°Brix		Pol (%)		RS (%)	
M. arenaria	17.75	b*	12.27	b	1.26	a
M. javanica	18.21	b	12.63	b	1.21	a
M.incognita	17.95	b	12.00	b	1.39	a
Control	21.53	a	18.05	a	0.76	b
CV %)	19.18		15.67		19.52	

*** Averages followed by the same letters in the column do not differ from each other by the Tukey test at 5% probability. CV: coefficient of variation.**

directly reflects the yield potential of the crop, according to the negative correlation between reproduction factor and leaf chlorophyll index (R=-0.75 to -0.85). Thus, the smaller the leaf area and leaf chlorophyll index value, the lower the interception efficiency, the conversion of intercepted radiation into phytomass, and the efficiency of assimilated partition to the plant part of economic interest (Forsthofer et al. 2006). The reduction in the physiological activity of the main carbohydrate producing sources, caused by the reduction of the leaf area in the reproduction phase, interferes in the redistribution of photoassimilates within the plant, thus altering the speed and intensity of leaf senescence and the patterns of carbohydrate accumulation in culms (Uhart & Andrade 1995).

Regarding the interference of root-knot nematode in the concentration of total soluble solids in the water of sugarcane plants infected, there was a negative correlation (R=-0.65), with an average reduction of 3.5 °Brix compared to the control without nematodes.

Figure 1. Fresh mass of culms of sugarcane plants inoculated or not with *Meloidogyne* spp. a) control (no *Meloidogyne* spp.), b) *M. incognita*, c) *M. javanica*, d) *M. arenaria*.

On the other hand, the reduction of Pol % and the increase of reducing sugars in the broth of sugarcane plants infected by *Meloidogyne* spp. can lead to quality losses in both alcohol and sugar production. Pol % measures the concentration of sucrose obtained in a solution, and the higher this content, the higher the recovery of sugar or ethanol at the end of the industrial process (Consecana 2006). The reducing sugars is formed by glucose and fructose, and its presence at high rates directly and negatively influences the quantity and quality of the product (Fernandes 2003). In another pathosystem, the inoculation of coffee plants with *Meloidogyne exigua* and *Meloidogyne paranaensis* affected the carbohydrate content in non-infected tissues, showing that the nematode can act as a metabolic drain not only in the gall or thickening region but also in areas far from the infection point (Goulart et al. 2019). This change in carbohydrate partition seems to be probably related to the nematode species and the population level (Carneiro et al. 1999).

The increase in the contents of neutral detergent fiber, acid detergent fiber, lignin, cellulose, and hemicellulose observed in plants inoculated with *Meloidogyne* spp. directly reflects on the digestibility of sugarcane, when these are destined for animal feed. Thus, neutral detergent fiber contents are important for choosing the genotypes of sugarcane with this purpose. Corrêa et al. (2003) studied the potential of sugarcane in diets for dairy cattle. These authors observed a decrease in the consumption of the diet containing sugarcane from the second week onward, evidencing the filling of the digestive tract with neutral detergent fiber of low digestibility. The acid detergent fiber is contained in the neutral detergent fiber as it represents the cellulose and lignin fractions, the

latter being the non-digestible fraction of the plant. Thus, the higher the acid detergent fiber content, the lower the quality and digestibility of sugarcane. In this regard, the infection of sugarcane plants with the root-knot nematode, in addition to reducing green mass production, may also interfere with the digestion and utilization of sugarcane by the animal, resulting in losses in milk or meat production.

The use of nematicides is the most commonly used control strategy in infected sugarcane plantations to reduce the damage caused by root-knot nematodes, thus contributing to the increase in sugarcane productivity, especially at the time of harvest (Dinardo-Miranda et al. 1995). A study on the productivity of varieties in fields infested by *M. javanica* showed that the use of nematicide resulted in productivity increases of about 15% for two susceptible genotypes, SP79-1011 and RB72454 (Dinardo-Miranda et al. 1995). Another study showed that areas infected by *M. incognita* treated with nematicides resulted in a reduction in populations of this species and led to average productivity increases of 50%, reaching 118% for the SP71-799 genotype (Novaretti et al. 1985). Garcia et al. (1997), also working in areas infested by *M. incognita*, observed increases in productivity of up to 40% due to the application of nematicides, with the RB72454 genotype showing an increase of 30%. Thus, the combined use of a nematicide with a variety tolerant to *Meloidogyne* spp. could provide a reduction in the number and amount of nematicide applied. However, depending on the level of crop infestation and the form of nematicide application, the effect of this product may be limited, besides posing severe risks to human health and the environment (Gomes et al. 2016).

Allied to nematicides, the use of products with pyraclostrobin could be an alternative since studies carried out in areas infested with *M. incognita* have shown an increase in agricultural productivity, probably by inducing resistance (Chaves et al. 2016). Another possible management strategy is the use of rotation with inadequate host species, such as peanut, rattlepod, pigeon pea, deer-eye bean, and lablab-bean, depending on the target nematode, during the period of cane field renewal (Stirling et al. 2011). In addition to this management strategy, the incorporation of organic waste, soil tillage and biological control (Cadet et al. 2004) seem to be good alternatives for the infested areas, aiming to reduce the initial population of the nematodes and consequently the damage caused, in line with the findings observed in this study.

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