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SCIENTIFIC ARTICLE
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Reaction of sugarcane genotypes to root-knot nematode parasitism (*Meloidogyne javanica* and *Meloidogyne incognita*)

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ABSTRACT
Root-knot nematodes (*Meloidogyne* spp.) can cause a reduction up to 50% in the production of sugarcane (*Saccharum* spp.). Genetic resistance is considered an important component in the management of nematodes. Therefore, the aim of this study was to evaluate the susceptibility of 12 sugarcane genotypes when subjected to parasitism by *Meloidogyne javanica* and *Meloidogyne incognita* under controlled conditions in a plant growth chamber. Four experiments were carried out in randomized blocks with 12 treatments and four replicates for *M. javanica* and three replicates for *M. incognita*. The treatments were the sugarcane genotypes: RB966928 (susceptibility pattern), RB026842, RB036168, RB036145, RB036065, RB966229, RB036066, RB036068, RB046209, RB036163, RB036153, and RB036059. Each repetition consisted of a 2-L pot with previously autoclaved substrate (1:1 of sand and soil), and a pre-sprouted seedling was transplanted. From pure populations of *M. javanica* and *M. incognita*, approximately 2,000 eggs and eventual J_2 were inoculated per pot. After 120 days, the final population of nematodes in the root and soil was counted using an optical light microscope and Peters slide, and the reproduction factor (RF) was calculated. Plants that presented RF < 1 were considered resistant, and plants that presented RF > 1 were considered susceptible. Among the 12 genotypes evaluated, 66% were susceptible to parasitism by *M. javanica*. The genotypes RB046209, RB036163, RB036153, and RB036059 were classified as resistant to *M. javanica* parasitism. For the species *M. incognita,* the susceptible genotypes represented 75% of the total, and the resistant genotypes were RB036163, RB036153, and RB036059.

Keywords: *Saccharum* spp.; nematode; genetic resistance; susceptibility; resistant.

INTRODUCTION

Sugarcane (*Saccharum* spp. L. hybrid) is a semi-perennial monoculture adapted to tropical and subtropical climates (MORAIS et al., 2015). Sugarcane plants cultivated commercially are mostly hybrids resulting from the backcrossing of several *Saccharum* species, mainly *S. officinarum* and *S. spontaneum* (FIGUEIREDO, 2010). They are versatile crops that generate several products and by-products, especially sugar and ethanol (MORAIS et al., 2015).

Currently, Brazil is the largest sugarcane producer in the world, followed by India, China, Thailand, Pakistan, and Mexico (FAO, 2020). With a planted area of 8,127.7 hectares, Brazil produced 572,874.9 tons in the 2022/23 harvest. However, crop production can be affected by environmental weather conditions, such as drought and frost, and by weeds, pests, diseases, among others (CONAB, 2022).

Diseases in the sugarcane crop caused by nematodes are of great importance because they can reduce production by up to 50% in highly susceptible cultivars with high nematode population levels (DINARDO-MIRANDA, 2005). Among

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the nematodes that affect crop production in Brazil, the root-knot nematodes *Meloidogyne javanica* (Treub) (Chitwood 1949) and *M. incognita* (Kofoide & White) (Chitwood 1949) stand out. The average reduction in productivity caused by *M. javanica* and *M. incognita* in susceptible cultivars is 20% and 40%, respectively (DINARDO-MIRANDA, 2005).

The genus *Meloidogyne* comprises the most important phytonematodes in the world (JONES et al., 2013). The characteristic symptoms of nematodes of the genus *Meloidogyne* are root thickenings called galls. They are formed because of cellular hypertrophy and hyperplasia in the vascular cylinder (JONES et al., 2013; FERRAZ, 2018). However, galls on the sugarcane root system, when formed, are rare and small. They are predominantly found at the tips of roots and are therefore difficult to detect (DIAS-ARIEIRA et al., 2010b; FERRAZ; BROWN, 2016). Other symptoms that can be observed in sugarcane fields are changes in the agronomic characteristics of cultivars, such as internode length, tiller height, and stem thickness (FERRAZ; BROWN, 2016).

Among the methods for phytonematode control in sugarcane, cultural (BERRY et al., 2011; SANTANA-GOMES et al., 2019), biological (CARDOZO; ARAÚJO, 2011; FERREIRA et al., 2017; MAZZUCHELLI et al., 2020), chemical (DINARDO-MIRANDA et al., 2008; DIAS-ARIEIRA et al., 2010a), and genetic resistance (DINARDO-MIRANDA et al., 1995; DIAS-ARIEIRA et al., 2010a; DINARDO-MIRANDA; FRACASSO, 2010; SILVA et al., 2012; SILVA et al., 2016; BELLÉ et al., 2017; THOMAZELLI et al., 2020) stand out. The application of biological and chemical products has shown low efficiency, mainly because the period of action of the product after application is very short compared with the fast life cycle of nematodes (FERREIRA et al., 2017), and the number of nematodes tends to return at high levels after 90 to 120 days after chemical or biological treatment (SILVA et al., 2016). In addition, the semi-perennial characteristic in which the sugarcane field can be grown for several years with little disturbance to the soil facilitates the increase in the nematode population in just two or three cultivation cycles, which makes cultural management difficult and results in a decline in productivity in subsequent harvests (JAYAKUMAR; GANAPATHY, 2020).

The use of resistant cultivars is an efficient, non-polluting, and cost-effective control measure. In Brazil, genotypes were tested for root-knot nematode parasitism resistance in the states of Pernambuco (SILVA et al., 2012; SILVA et al., 2016), São Paulo (DINARDO-MIRANDA et al., 1995; DINARDO-MIRANDA; FRACASSO, 2010; THOMAZELLI et al., 2020), Paraná (DIAS-ARIEIRA et al., 2010a), and Rio Grande do Sul (BELLÉ et al., 2017), demonstrating differences in the resistance/susceptibility of the genotypes in relation to the root-knot nematode.

The Inter-University Network for the Development of the Sugar-Energy Sector (RIDESA) breeding program plays an important role in the development of sugarcane genotypes aimed at increasing productivity, in addition to the search for disease-resistant genotypes (DAROS et al., 2016). Testing genotypes for nematode parasitism resistance is a complementary and indispensable strategy for this program. Therefore, the objective of this study was to evaluate the reaction of 12 sugarcane genotypes from RIDESA to the parasitism of *M. javanica* and *M. incognita*.

MATERIAL AND METHODS

This study consisted of four experiments. The first and second experiments (E1 and E2) were carried out from September 2020 to January 2021, and the third and fourth experiments (E3 and E4), from November 2020 to March 2021. The experiments were conducted in a plant growth chamber (Fitotron, Instalafrio) under controlled conditions (28°C ± 2), at the Plant Science and Plant Protection, Universidade Federal do Paraná (UFPR). RIDESA produced the pre-sprouted seedlings in Paranavaí, PR, Brazil, at the RIDESA/UFPR experimental station.

The experiments were conducted in a randomized block design for operational reasons in the evaluation of the experiments, with 12 treatments consisting of the following sugarcane genotypes: RB966928, RB026842, RB036168, RB036145, RB036065, RB966229, RB036066, RB036068, RB046209, RB036163, RB036153, and RB036059. Based on the results obtained from DIAS-ARIEIRA et al. (2010b) and BELLÉ et al. (2017), genotype RB966928 was considered as the susceptibility standard.

Experiments E1 and E3 evaluated the resistance of cultivars to *M. javanica* parasitism and had four replications. Experiments E2 and E4 evaluated the resistance of the genotypes to the parasitism of *M. incognita* with three replications. Each replicate consisted of a 2-L vase with soil previously autoclaved at 120° C for 1 hour, in a 1:1 proportion of sand and soil.

Pre-sprouted 80-day-old seedlings were transplanted into pots. After seven days of transplanting, they were fertilized with 50-120-100 kg·ha^{-1} (N-P-K), as recommended by PAULETTI and MOTTA (2019), for the average expected productivity of plant cane, thus totaling 0.11 g of urea, 0.29 g supertriple, and 0.17 g KCl per pot. Twenty days after transplantation, each pot was inoculated with approximately 2,000 eggs and any J2 from the pure population of *M. javanica* and *M. incognita*, which were produced in tomato plants of the Santa Clara cultivar kept in a greenhouse for 60 days.

The suspensions of *M. javanica* and *M. incognita* specimens were obtained using the centrifugal flotation technique in sucrose solution (COOLEN; D'HERDE, 1972). The suspension was calibrated and applied by depositing 1 mL of the suspension containing the eggs and any J2 of *M. javanica* and *M. incognita* in three equidistant holes around the plants with a depth of 4 cm previously made with the aid of a Falcon tube. To test the viability of the inoculum, the same amount was inoculated into 25-day- old 'Santa Clara' tomato seedlings susceptible to *Meloidogyne*. Four replicates were performed for each species.

One hundred and 20 days after inoculation, the plants were removed from the pots, and the roots were collected, washed, and placed on absorbent paper to remove excess water. They were weighed and cut into fractions of 2 to 5 cm. A 5-g sample taken from random spots of clean roots was separated and destined for nematode extraction. Root fragments were processed in a blender and floated in sucrose (COOLEN; D'HERDE, 1972). When preparing the soil for extraction, the contents of the pots were poured into a tray, homogenized, and 50 cm^3 of the soil was removed at random spots. The aliquot was subjected to extraction following JENKINS' (1964) method.

Using a Peters chamber, the number of eggs and J2 in the roots and soil was counted under an optical light microscope at 40x magnification. To obtain the final population, the nematodes counted in the 5-g root suspension were multiplied by the total mass of the root system and added to those in the soil. The reproduction factor (RF) was evaluated using the initial and final population ratio (RF = final population/initial population). The reactions of the sugarcane genotypes were classified according to the RF value, considering resistant plants that presented RF < 1 and susceptible plants with RF > 1 (OOSTENBRINK, 1966).

The data from the experiments were submitted to tests of normality and homogeneity of variance, in a 12 × 2-factorial scheme, with the first factor being the genotypes and the second factor being the experiment time (repetition) for each evaluated species. Analysis of variance (ANOVA) was performed, followed by the Scott-Knott's test at a 5% probability level using R software version 4.0.4 (R Core Team).

RESULTS

The inoculum was proven viable, presenting in tomato plants an average RF of 3.62 (standard deviation = 1.4) for *M. javanica* and 6.61 (standard deviation = 3.2) for *M. incognita*.

There was no significant interaction between the factors genotypes and repetition of experiments, and there was no effect of experiment time. The two experiments for each species of root-knot nematodes were grouped.

Regarding *M. javanica* parasitism, genotypes RB966928, RB026842, RB036168, RB036145, RB036065, RB966229, RB036066, and RB036068 showed susceptibility (RF > 1). On the other hand, the RB046209, RB036163, RB036153, and RB036059 genotypes were classified as resistant (RF < 1) (Table 1). The RB966928 genotype, used as a susceptibility standard, showed the highest reproduction factor for *M. javanica*, with RF = 3.46, whereas the RB046209 genotype presented the lowest reproduction factor (0.49) (Table 1).

Table 1. Final population and reproduction factor of *Meloidogyne javanica* and *Meloidogyne incognita* and reaction of the different sugarcane genotypes to nematodes.

Genotype	*Meloidogyne javanica*			*Meloidogyne incognita*		
	FP	RF	R#	FP	RF	R
RB966928	6,920.16* a	3.46***	S	3,746.15**** a	1.87	S
RB026842	5,627.87** a	2.81	S	6,511.57 a	3.25	S
RB036168	4,938.66 a	2.46	S	3,027.38 a	1.51	S
RB036145	4,278.24 a	2.13	S	3,453.07 a	1.72	S
RB036065	3,675.00 a	1.83	S	2,154.33 a	1.07	S
RB036229	3,649.57 a	1.82	S	3,782.55 a	1.89	S
RB036066	2,808.27 b	1.40	S	2,379.31 a	1.18	S
RB036068	2,377.95 b	1.18	S	2,058.09 a	1.02	S
RB046209	996.64 b	0.49	R	2,432.44 a	1.21	S
RB036163	1,873.35 b	0.93	R	1,913.44 a	0.95	R
RB036153	1,905.58 b	0.95	R	1,506.14 b	0.75	R
RB036059	1,252.75 b	0.62	R	472.72 b	0.23	R
CV		69.96%			93.84%	

*Means followed by the same line do not differ in the column by the Scott-Knott's test at 5%; * untransformed data; ***average of four repetitions; ****average of three repetitions; CV: coefficient of variation; FP: final population; RF: reproduction factor = final population/initial population (Pi = 2,000); R#: reaction; S: susceptibility (RF > 1); R: resistance (RF < 1).

Regarding *M. incognita* parasitism, nine of the genotypes (RB966928, RB026842, RB036168, RB036145, RB036065, RB966229, RB036066, RB036068 and RB046209) tested showed susceptibility (RF > 1). Genotypes RB036163, RB036153, and RB036059 were classified as resistant (RF < 1) (Table 1). Genotype RB026842 showed the highest RF (3.25), and genotype RB036059 had the lowest RF value (0.23).

DISCUSSION

In this study, variation in the reaction of 12 sugarcane genotypes was observed when subjected to parasitism by *M. javanica* and *M. incognita*. These results are essential to help direct the choice of genotype to be used in the management of sugarcane in areas infested by root-knot nematodes.

Meloidogyne javanica is a widely spread species in sugarcane areas. In northwest Paraná, it is the predominant species, with an incidence of 46% in the areas sampled by SEVERINO et al. (2010). In a survey in the three mesoregions of the state of Paraná, in 2020, Meloidogyne spp. was frequent in 70% of the root samples (MARTINHA et al., 2022). In addition, NOVARETTI (1995) found that eight individuals per gram of roots indicate a high population density and can cause damage to the crop. In a study carried out in Paraná, in which the reaction of sugarcane genotypes with the acronyms RB and CTC to the parasitism of *M. javanica* and *M. incognita* was tested. DIAS-ARIEIRA et al. (2010b) observed that the evaluated genotypes showed susceptibility to both species. As in this study, the species *M. javanica* showed higher RF values.

The RB046209, RB036163, RB036153, and RB036059 genotypes tested in this study showed promising results regarding resistance to *M. javanica*. However, it is important to emphasize that this behavior can vary in the field. For example, in experiments carried out in different regions with variation in temperature, humidity, and soil type, DINARDO-MIRANDA et al. (2019) found that the reproduction and development of nematodes are directly affected by temperature, and soil moisture, which also interfere with dormancy, diapause, and the nematode life cycle.

Another experiment by DINARDO-MIRANDA et al. (1995) tested 18 genotypes in areas naturally infested by *M. javanica* and observed that all provided reproduction of the nematode. However, 12 genotypes were considered susceptible. Different reproduction rates may, in part, be associated with the genetic factor of the host, conferring resistance or susceptibility, as well as with the genetic characteristics of nematode populations. Nematodes that reproduce by mitotic parthenogenesis, such as *M. javanica*, can adapt quickly and overcome host resistance (CASTAGNONE-SERENO, 2006). This was observed in studies by BELLÉ et al. (2017), who evaluated the reaction of RB group genotypes to *M. javanica* and found that all genetic materials tested were susceptible to the root-knot nematode. As in the present study, the RB966928 genotype has been identified in several studies as a good host for *M. javanica* (BELLÉ et al., 2017; DIAS-ARIEIRA et al., 2010b; DINARDO-MIRANDA et al., 2019).

Regarding the *M. incognita* species, the RB036163, RB036153, and RB036059 genotypes showed resistant behavior. In Brazil, in addition to these genotypes, the CTC-17 genotype and RB041443 have already been reported to be resistant to *M. incognita* (DIAS-ARIEIRA et al., 2010b). However, SILVA et al. (2016), when testing 23 genotypes, found that all were susceptible to M. incognita.

Research carried out in other countries has proven the susceptibility of sugarcane genotypes to root-knot nematodes. In Nigeria, SALAWU (1992) evaluated the reaction of 12 genotypes to *M. incognita* and observed that all materials behaved as highly susceptible, even those in which shoot and root masses were high.

DIAS-ARIEIRA et al. (2010a) point out that, although the evaluation method by the RF presents some limitations, it is the most viable for use in sugarcane, since in this host the formation of visible galls does not always occur, which makes it difficult to use scales based on the number of galls or egg mass.

This study demonstrated the presence of resistant genotypes to *M. javanica* and *M. incognita*. Genotypes with susceptible reactions can be used preferentially in areas without the presence of the nematode. These results are essential to help both genetic improvement programs in the development of genotypes and to direct the use of these genotypes in areas infested or not with root-knot nematodes.

CONCLUSIONS

Among the 12 genotypes evaluated, 34% were resistant to *M. javanica* parasitism, whereas the *M. incognita* species resistant genotypes represented 25% of the total.

Genotypes RB036163, RB036153, and RB036059 showed resistant behavior in both species.

AUTHORS' CONTRIBUTIONS
Conceptualization: Duarte, H.S.S.; Ruaro, L.; Oliveira, R.A.; Martinha, D.D. **Data curation:** Martinha, D.D.; Silva, M.C.C.; Rocha, M.G.C.; Hahn, M.H. **Formal analysis:** Martinha, D.D.; Candido, M.C.; Rocha, M.G.C.; Quadros, L.P. **Funding acquisition:** Oliveira, R.A. Investigation: Martinha, D.D. **Methodology:** Martinha, D.D.; Ruaro, L.; Candido, M.C.; Rocha, M.G.C.; Quadros, L.P.; 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.; Hahn, M.H.; Ruaro, L. **Visualization:** Martinha, D.D.; Silva, M.C.C.; Hahn, M.H.; Ruaro, L.; Oliveira, R.A.; Duarte, H.S.S. **Writing – original draft:** Martinha, D.D. **Writing – review & editing:** Martinha, D.D.; 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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The authors certify that they have no commercial or associative interest that represents a conflict of interest in connection with the manuscript.

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