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PLANT PATHOLOGY & NEMATOLOGY

Comparative Pathogenicity of *Meloidogyne enterolobii* and *Meloidogyne incognita* in Cotton Cultivars

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ABSTRACT

***Meloidogyne enterolobii* (*Me*) is a nematode that has recently been observed in cotton. Initial observations indicate that cotton cultivars resistant to *Meloidogyne incognita* (*Mi*) are susceptible to *Me*, but the reproductive capacity and ability to cause damage by *Me* compared to *Mi* is unknown. The objective of this study was to assess the reproductive capacity and ability to cause damage by *Mi* and *Me* at different population densities in two cotton cultivars. The study included two greenhouse experiments in which two cotton cultivars, resistant and susceptible to *Mi*, were inoculated with six increasing population densities of *Me* and *Mi*. The genetic resistance of cotton to *Mi* given by quantitative trait loci of the q-Mi11 and q-Mi14 genes is ineffective against *Me*. The *Mi*-resistant cotton cultivar showed a similar gall index to the *Mi*-susceptible cultivar when inoculated with *Me*. The mean gall index with *Me* was significantly higher than that with *Mi* on the *Mi*-resistant cotton cultivar. Based on the number of nematodes per root mass and the reproductive factor, *Me* showed a higher reproductive capacity than *Mi* on cotton. Increasing *Me* population densities linearly reduced the height and dry matter production of aboveground biomass in the *Mi*-resistant cotton cultivar. However, neither *Meloidogyne* species affected leaf chlorophyll content and photosynthetic rate of the cotton plants.**

Meloidogyne incognita (Kofoid & White) Chitwood (*Mi*) is the main root-knot nematode affecting cotton crops in Brazil (Galbieri and Asmus, 2016), occurring in 25% of crop production areas in the state of Mato Grosso (Galbieri et al., 2016), and 37% of the planted area in the state of Bahia (Perina et al., 2018). Several crop-breeding programs in Brazil have worked on the introgression of nematode resistance via molecular markers (Gutiérrez et al., 2010), as well as on the development of cotton cultivars adapted to the tropical conditions of the Brazilian Cerrado associated with transgenic events to increase tolerance to herbicides and resistance to Lepidoptera. The release of the first cotton cultivar resistant to *Mi* for commercial use in Brazil took place in the 2017/18 crop year (Belot et al., 2020). In 2022/23, approximately 198,000 hectares were sown with cotton cultivars resistant to *Mi* in Brazil (Galbieri et al., 2023), of a total of 1.66 million hectares (CONAB, 2024). However, in 2019 the species *Meloidogyne enterolobii* Yang and Eisenback (Sin. *M. mayaguensis* Rammah and Hirchmann) (*Me*) was detected in the state of Minas Gerais parasitizing the cotton cultivar IMA 5801B2RF (Galbieri et al., 2020), which has resistance to *Mi* (Belot et al., 2020). Since this first report, *Me* was found in cotton crops geographically far from the first occurrence, including western Bahia (Souza et al., 2022), and in 2023, northwestern Mato Grosso (unpublished data, Mato Grosso Cotton Institute).

It is noteworthy that *Me* was first detected in Brazil in 2001, in the states of Pernambuco and Bahia, damaging guava crops (Carneiro et al., 2001), but populations of *Me* from guava crops were not capable of parasitizing cotton (Carneiro et al., 2006b). However, in recent years, high populations of *Me* have significantly damaged cotton crops in Brazil and in different parts of the world, including the U.S., specifically North Carolina (Ye et al., 2013), where it is also increasing in occurrence in sweet potato, tobacco, and soybean crops (Schwarz et al., 2020). Several U.S. states have imposed quarantine measures on the transport of soil and plants originating from infested areas (Ye et al., 2021).

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Temperatures close to or at 30 °C favor *Me* (Veloso et al., 2022). Available data on the global occurrence of *Me* indicates that global potential distribution of *Me* occurs on all continents except Antarctica, and most suitable areas are concentrated in Africa, South America, Asia, and North America between latitudes 30° S and 30° N (Pan et al., 2023). The planting sequence of susceptible crops, including soybeans and cotton, creates an ideal scenario for the development of this nematode in the Brazilian Cerrado. According to Collett et al. (2023), *Me* is the most competitive species having a shorter life cycle with greater multiplication potential (generations) compared to *Mi* and *M. javanica* (Treub). Field observations showed that *Me*-induced galls on cotton are larger than those caused by *Mi* (Ye et al., 2013; Galbieri et al., 2020). However, the comparative ability of *Mi* and *Me* to reproduce and cause damage to cotton remains unknown. The objective of this study was to assess the reproductive capacity and ability to cause damage by *Mi* and *Me* at different population densities on two cotton cultivars.

MATERIALS AND METHODS

The study included two greenhouse experiments in Dourados, state of Mato Grosso do Sul (MS), Brazil (experiment 1), and in Primavera do Leste, state of Mato Grosso (MT), Brazil (experiment 2). The reproductive capacity and ability of *Mi* and *Me* to cause damage at different population densities on two cotton cultivars were assessed: IMA 5801B2RF (Instituto Mato-Grossnse do Algodão), which carries quantitative trait loci (QTL) of the q-Mi11 and q-Mi14 genes that give resistance to *Mi*; and TMG 44B2RF (Tropical Melhoramento Genético), which is susceptible to *Mi* (Galbieri et al., 2023). The experiments followed a completely randomized factorial design (inoculum densities x *Meloidogyne* species x cotton cultivars), with six replications in experiment 1 and seven replications in experiment 2. The *Mi* and *Me* populations used in the experiments were obtained from parasitized cotton roots collected in Primavera do Leste, MT, and Paracatu, state of Minas Gerais, respectively. They were characterized and analyzed in other studies by Galbieri et al. (2021), who used *Mi*; and Galbieri et al. (2020) and Vessiani et al. (2023), who used *Me*. The samples were kept and multiplied in Rutgers tomato plants.

Experiment 1. On 25 July 2022, three delinted and untreated seeds of each cotton cultivar were sown in 200-mL plastic cups containing 180 mL of substrate composed of soil and sand (1:1) disinfected by solarization. After 10 d, the plants were thinned so that only one remained per cup. Twenty days after thinning, the plants were inoculated with 5 mL of suspensions containing 0, 300, 1000, 3000, 9000, and 27,000 eggs and some second-stage juveniles (J_2) of *Mi* or *Me*, which were deposited in two holes approximately 2 cm deep, aside 1 cm from the stem. For inoculum production, the nematode populations were multiplied in Rutgers tomato plants and extracted from the roots according to Hussey and Barker as modified by Bonetti and Ferraz (1981), followed by centrifugation in sucrose. Eight days after inoculation (DAI), the plants and substrate were carefully removed from the plastic cups and transplanted into 2.25-L ceramic pots containing 2 L of substrate (68.3% sand, 4.8% silt, and 26.9% clay) disinfected by solarization and fertilized with 3 g of NPK (20-20-20). The pots were drip irrigated daily in three watering cycles, totaling approximately 200 mL of water/day/plant. Top dressing with 2 g of urea and 2 g of KCl per pot was applied 44 DAI. During the experimental period, the plants were sprayed with methomyl (Lannate®BR, Corteva AgrisScience do Brasil Ltda.) 34 and 44 DAI to control aphids.

Plant height was measured 20 and 40 DAI as the distance between substrate and stem apical meristem. Total chlorophyll content (ng cm^{-2}) of the first fully expanded leaf of each plant was measured with a portable chlorophyll meter (ChlorofiLOG®, Falker Automação Agrícola, Brazil) 57 DAI. Leaf photosynthetic rate (µmol CO2 cm^{-2}) was determined 63 DAI using a LCpro-SD® portable meter (ADC BioScientific LTD, UK) with artificial light (1,000 µmol $m^{-2}s^{-1}$), at a wavelength between 440 and 485 nm. The central part of the last well-formed and expanded leaf of each plant remained in the analysis chamber for 3 to 5 min. until the reading stabilized.

At 70 DAI, the cotton plants were carefully separated from the substrate by cutting them at the base of the stem. The aboveground biomass was placed in paper bags and dried in a drying oven for 7 d at 55 °C for subsequent determination of total shoot dry matter (SDM). Roots were carefully separated from the substrate under running water, placed on absorbent paper for approximately 20 min. to absorb excess moisture, and weighed to determine root fresh weight. They were then analyzed for gall index (GI) according to Taylor and Sasser (1978) and processed for egg extraction (Coolen and D'Herde, 1972). The fresh root mass and the number of eggs extracted

from each root system were used to estimate the number of nematodes per gram of root (NGR) and the reproduction factor (RF), which is the total number of eggs extracted from the root/number of eggs inoculated.

Data were subjected to analysis of variance by the F-test ($p \leq 0.05$). When the effect of the quantitative variable (nematode population density) was significant, analysis of variance was carried out for the polynomial regression of treatment effects to indicate, among the statistically significant functions, the one with the best fit to data (highest determination quotient, R^2). To comply with the normal distribution, NGR and RF data were transformed into log (x+1) prior to analysis of variance. All statistical analyses were carried out using the AgroEstat software (Barbosa and Maldonado Júnior, 2015).

Experiment 2. Experiment 2 was carried out in Primavera do Leste, with a few methodological adjustments to adapt to the procedures routinely used by the local research team. Thus, the plastic pots measured 15 cm in diameter and contained 2.4 L of substrate composed of Vivatto® (Technes Agrícola Ltda.) + sand (1:1), with a final composition of 85% sand, 5% silt, and 10% clay, sterilized in an autoclave at 120 °C for 15 minutes, and fertilized with 2 g of NPK (20-20-20). On 7 November 2022, three seeds were sown directly into these pots, with thinning 10 d later to keep one plant per pot. The seeds were inoculated 15 d after sowing (same procedure and quantity as in experiment 1). Top dressing with 2 g of Osmocote (14-14-14) per pot was applied 15 DAI. During the experimental period, the plants were sprayed with thiamethoxam (Actara®, Syngenta Proteção de Cultivos Ltda.) 29 and 44 DAI to control aphids and whitefly. Plant height was measured 20 and 40 DAI. Fresh weight, SDM, and GI were measured 70 DAI, and roots were processed for nematological analysis. The other methodological procedures and analyses were identical to experiment 1. For statistical analyses, NGR and RF data were transformed into log (x+1).

RESULTS AND DISCUSSION

The summary of the analysis of variance for all variables evaluated is shown in Tables 1 (experiment 1) and 2 (experiment 2). There was no significant difference in the height of plants inoculated with *Mi* or *Me*, but there was a significant interaction between population density and nematode species. The effect of *Meloidogyne* population density on plant height was observed only with *Me* infecting cultivar IMA 5801B2RF in experiment 1. The linear model showed the best fit to data both at 20 and 40 DAI (Fig. 1).

Leaf chlorophyll content values ranged from 35.3 ng cm^{-2} in TMG 44B2RF to 41.2 ng cm^{-2} in IMA 5801B2RF, and photosynthetic rate ranged from 17.1 µmol CO_2 cm^{-2} in TMG 44B2RF to 19.7 µmol CO_2 cm^{-2} in IMA 5801B2RF. Both variables were not affected by the *Meloidogyne* species or nematode population density.

Increasing *Me* population densities reduced the SDM of cultivar IMA 5801B2RF ($y = 19.9100 - 0.0002x$; $R^2 = 0.78$; $p = 0.0028$) in experiment 1 (Fig. 2). In both experiments, cultivar IMA 5801B2RF produced a larger SDM than TMG 44B2RF. There was no significant difference in SDM between nematode species.

Primary damage caused by *Meloidogyne* species to the cotton root system was evident by GI, which showed differences between cultivars, species, and population densities (Table 3, Figs. 3 and 4). In both experiments, the *Mi* GI was significantly lower in cultivar IMA 5801B2RF than TMG 44B2RF. However, when the plants were inoculated with *Me*, GI was similar between cultivars in experiment 2, but higher in cultivar IMA 5801B2RF in experiment 1. Regardless of cultivar, the GI in plants inoculated with *Me* was significantly higher than in plants inoculated with *Mi* in experiment 2. However, in experiment 1, *Me* GI was significantly higher than *Mi* GI in cultivar IMA 5801B2RF and lower in cultivar TMG 44B2RF. The effect of population density on GI exhibited difference between species and cultivars (Tables 1 and 2, Figs. 3 and 4). In both experiments, cultivar IMA 5801B2RF tolerated high *Mi* population densities, showing low GI even at the highest nematode density, but high GI when inoculated with *Me*. Increasing *Me* and *Mi* population densities had a similar effect on GI in cultivar TMG 44B2RF (Figs. 3 and 4).

The reproduction of *Mi* and *Me* in cotton cultivars was assessed by estimating NGR and RF. *M. incognita* reproduction was significantly lower in cultivar IMA 5801B2RF in both experiments (Tables 4 and 5), as demonstrated by the smaller NGR. Plants inoculated with *Me* showed no significant difference in NGR between cultivars. NGR was significantly higher when cultivar IMA 5801B2RF was inoculated with *Me*. However, there were no significant differences in

Table 1. Analysis of variance for main effects and interactions of variables evaluated in Dourados (experiment 1)

Plant Height 20 DAI			
Factors	**DF**	**MS**	**F[z]**
Cultivar (C)	1	1347.50	59.62**
Nematode (N)	1	35.5	1.57ns
Population (P)	5	72.3	3.20**
C x N	1	17.0	0.75ns
C x P	5	14.4	0.64ns
N x P	5	56.4	2.49*
C x N x P	5	3.0	0.13ns
Treatments	23	-	
Residue	120	22.6	
Total	143	-	
CV (%)	21.50		

Plant Height 40 DAI			
Factors	**GL**	**QM**	**F**
Cultivar (C)	1	4080.01	44.35**
Nematode (N)	1	70.14	0.76ns
Population (P)	5	194.32	2.11ns
C x N	1	92.64	1.01ns
C x P	5	79.91	0.87ns
N x P	5	230.04	2.50*
C x N x P	5	24.13	0.26ns
Treatments	23	-	
Residue	120	91.99	
Total	143	-	
CV (%)	20.29		

Leaf Chlorophyll Content			
Factors	**GL**	**QM**	**F**
Cultivar (C)	1	1196.58	26.83**
Nematode (N)	1	28.18	0.63ns
Population (P)	5	34.37	0.77ns
C x N	1	4.30	0.10ns
C x P	5	97.87	2.19ns
N x P	5	35.64	0.8ns
C x N x P	5	95.06	2.13ns
Treatments	23	-	
Residue	120	44.59	
Total	143	-	
CV (%)	14.86		

Photosynthetic Rate			
Factors	**GL**	**QM**	**F**
Cultivar (C)	1	154.92	42.00**
Nematode (N)	1	7.33	1.99ns
Population (P)	5	5.22	1.41ns
C x N	1	1.31	0.36ns
C x P	5	3.24	0.88ns
N x P	5	4.66	1.26ns
C x N x P	5	4.45	1.21ns
Treatments	23	-	
Residue	120	3.69	
Total	143	-	
CV (%)	10.59		

Shoot Dry Matter			
Factors	**GL**	**QM**	**F**
Cultivar (C)	1	136.07	6.89**
Nematode (N)	1	43.67	2.21ns
Population (P)	5	32.32	1.64ns
C x N	1	33.06	1.67ns
C x P	5	15.69	0.79ns
N x P	5	63.45	3.21**
C x N x P	5	9.53	0.48ns
Treatments	23	-	
Residue	120	19.74	
Total	143	-	
CV (%)	24.15		

Gall Index			
Factors	**GL**	**QM**	**F**
Cultivar (C)	1	22.56	66.85**
Nematode (N)	1	18.06	53.52**
Population (P)	5	40.46	119.89**
C x N	1	47.84	141.75**
C x P	5	1.59	4.73**
N x P	5	1.16	3.44**
C x N x P	5	2.27	6.74**
Treatments	23	-	
Residue	120	0.34	
Total	143	-	
CV (%)	25.58		

Continued

Table 1. continued

Nematodes per Gram of Root[y]			
Factors	**GL**	**QM**	**F[z]**
Cultivar (C)	1	22.62	357.29**
Nematode (N)	1	15.50	244.87**
Population (P)	5	12.17	192.17**
C x N	1	18.37	290.19**
C x P	5	1.13	17.94**
N x P	5	0.99	15.61**
C x N x P	5	1.02	16.11**
Treatments	23	-	
Residue	120	0.06	
Total	143	-	
CV (%)	19.85		

Reproduction Factor[y]			
Factors	**GL**	**QM**	**F**
Cultivar (C)	1	1.52	120.17**
Nematode (N)	1	0.12	9.63**
Population (P)	5	0.57	45.21**
C x N	1	0.79	62.33**
C x P	5	0.11	8.54**
N x P	5	0.02	1.78ns
C x N x P	5	0.07	5.57**
Treatments	23	-	
Residue	120	0.01	
Total	143	-	
CV (%)	51.57		

[z]ns, not significant; *, $p < 0.05$; **, $p < 0.01$
[y]For statistical analysis, the original data were transformed into log (x+1)

Table 2. Analysis of variance of the main effects and interactions of the variables evaluated in Primavera do Leste (experiment 2)

Plant Height 20 DAI			
Factors	**GL**	**QM**	**F[z]**
Cultivar (C)	1	1120.11	215.80**
Nematode (N)	1	1.21	0.23ns
Population (P)	4	2.43	0.47ns
C x N	1	2.31	0.45ns
C x P	4	2.45	0.47ns
N x P	4	5.22	1.01ns
C x N x P	4	5.31	1.02ns
Treatments	19	-	
Residue	120	5.19	
Total	139	-	
CV (%)	10.96		

Plant Height 40 DAI			
Factors	**GL**	**QM**	**F**
Cultivar (C)	1	2252.02	127.15**
Nematode (N)	1	13.51	0.76ns
Population (P)	4	10.64	0.60ns
C x N	1	8.02	0.45ns
C x P	4	12.42	0.70ns
N x P	4	24.78	1.40ns
C x N x P	4	8.33	0.47ns
Treatments	19	-	
Residue	120	17.71	
Total	139	-	
CV (%)	9.25		

Shoot Dry Matter			
Factors	**GL**	**QM**	**F**
Cultivar (C)	1	310.52	0.75ns
Nematode (N)	1	297.55	0.72ns
Population (P)	4	281.86	0.68ns
C x N	1	329.26	0.79ns
C x P	4	386.50	0.93ns
N x P	4	264.35	0.64ns
C x N x P	4	320.93	0.77ns
Treatments	19	-	
Residue	120	415.56	
Total	139	-	
CV (%)	62.55		

Gall Index			
Factors	**GL**	**QM**	**F**
Cultivar (C)	1	86.42	35.76**
Nematode (N)	1	122.58	50.72**
Population (P)	4	138.71	57.40**
C x N	1	81.78	33.84**
C x P	4	5.74	2.38ns
N x P	4	23.80	9.85**
C x N x P	4	17.61	7.29**
Treatments	19	-	
Residue	120	2.42	
Total	139	-	
CV (%)	56.68		

Continued

Table 2. continued

Nematodes per Gram of Root[y]			
Factors	**GL**	**QM**	**F**
Cultivar (C)	1	8.47	61.10**
Nematode (N)	1	9.33	67.37**
Population (P)	4	61.57	444.31**
C x N	1	4.64	33.50**
C x P	4	0.69	5.00**
N x P	4	0.61	4.44**
C x N x P	4	0.29	2.13ns
Treatments	19	-	
Residue	120	0.14	
Total	139	-	
CV (%)	14.21		

Reproduction Factor[y]			
Factors	**GL**	**QM**	**F**
Cultivar (C)	1	7.63	86.96**
Nematode (N)	1	4.72	53.76**
Population (P)	4	13.02	148.27**
C x N	1	2.46	28.07**
C x P	4	0.78	8.94**
N x P	4	0.41	4.68**
C x N x P	4	0.17	1.98ns
Treatments	19	-	
Residue	120	0.09	
Total	139	-	
CV (%)	31.74		

[z]ns, not significant; *, $p < 0.05$; **, $p < 0.01$

[y]For statistical analysis, the original data were transformed into log (x+1).

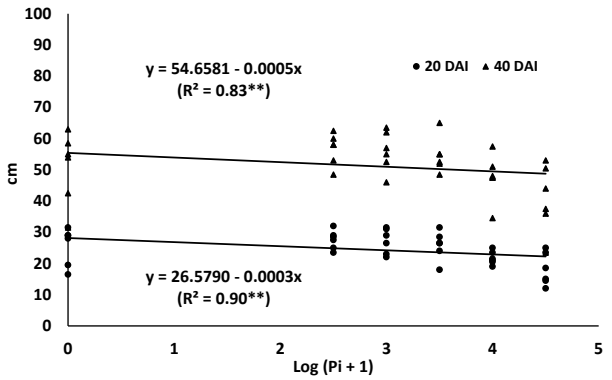

Figure 1. Plant height of cotton cultivar IMA 5801B2RF 20 and 40 days after inoculation (DAI) with increasing *Meloidogyne enterolobii* population densities (Pi), in Dourados (experiment 1).

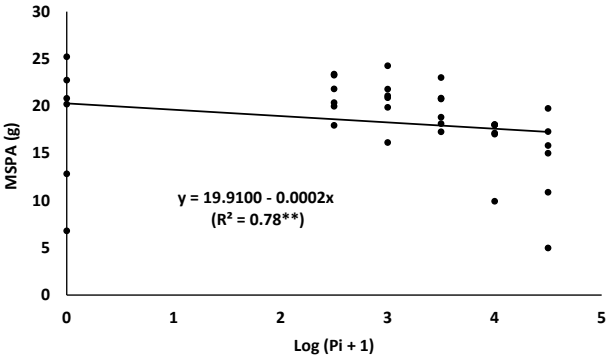

Figure 2. Shoot dry matter (SDM) of cotton cultivar IMA 5801B2RF inoculated with increasing *Meloidogyne enterolobii* population densities (Pi), in Dourados (experiment 1).

Table 3. Gall index (GI) on the roots of cotton cultivars IMA 5801B2RF and TMG 44B2RF inoculated with *Meloidogyne incognita* (*Mi*) or *Meloidogyne enterolobii* (*Me*), in Dourados (experiment 1) and Primavera do Leste (experiment 2)

	Experiment 1			Experiment 2		
	Mi	*Me*	Mean	*Mi*	*Me*	Mean
IMA 5801B2RF	0.94 aB[z]	2.81 aA	1.88 a	0.25 aB	3.66 aA	1.95 a
TMG 44B2RF	2.89 bA	2.44 bB	2.67 b	3.36 bA	3.70 aA	3.52 b
Mean	1.92 A	2.63 B		1.81 A	3.68 B	

[z]Means followed by the same lowercase letter in columns and uppercase letter in rows do not differ by the F-test (5%).

Table 4. Number of nematodes per gram[z] of root (NGR) (eggs and J_2) of the species *Meloidogyne incognita* (*Mi*) or *Meloidogyne enterolobii* (*Me*) in cotton cultivars IMA 5801B2RF and TMG 44B2RF, in Dourados (experiment 1) and Primavera do Leste (experiment 2)

	Experiment 1			Experiment 2		
	Mi	*Me*	Mean	*Mi*	*Me*	Mean
IMA 5801B2RF	0.9 bB[y]	125.8 aA	63.4 a	342.0 bB	3641.4 aA	1991.7 a
TMG 44B2RF	147.8 aA	153.1 aA	150.5 b	4134.1 aA	4673.2 aA	4403.7 b
Mean	74.4 A	139.5 B		2238.1 A	4157.3 B	

[z]Original means. For statistical analysis, the original data were transformed into log (x+1).

[y]Means followed by the same lowercase letter in columns and uppercase letter in rows do not differ by the F-test (5%).

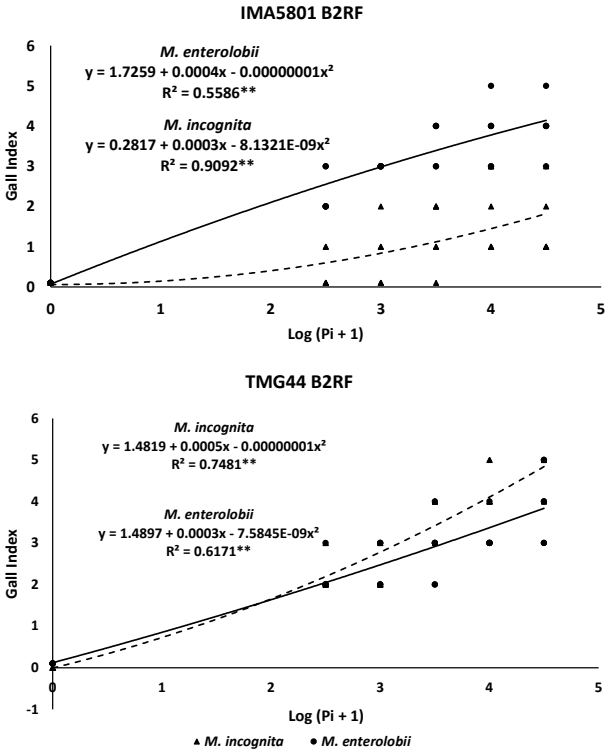

Figure 3. Gall index (GI) on the roots of cotton cultivars IMA 5801B2RF and TMG 44B2RF with increasing *Meloidogyne incognita* (solid line) or *Meloidogyne enterolobii* (dashed line) population densities (Pi), in Dourados (experiment 1).

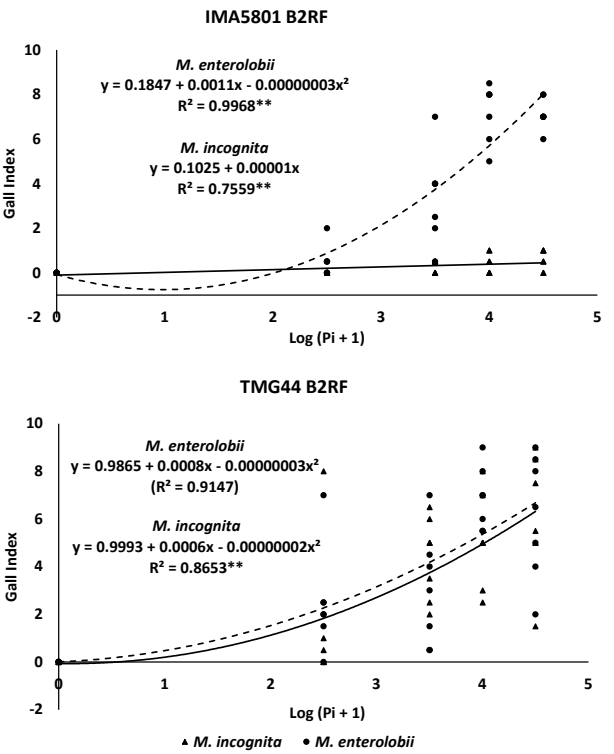

Figure 4. Gall index (GI) on the roots of cotton cultivars IMA 5801B2RF and TMG 44B2RF with increasing *Meloidogyne incognita* (solid line) or *Meloidogyne enterolobii* (dashed line) population densities (Pi), in Primavera do Leste (experiment 2).

NGR in cultivar TMG 44B2RF between species. The effect of population density on NGR differed between species and cultivars (Tables 1 and 2, Figs. 5 and 6).

Experiment 1 showed no significant differences in NGR between *Mi* population densities in cultivar IMA 5801B2RF. However, plants of the same cultivar inoculated with *Me* showed significant NGR differences between population densities, with a linear fit in experiment 1 ($y = 31.2759 + 0.01408x$; $R^2 = 0.9053$; $p = 0.0001$) and a quadratic fit in experiment 2 ($y = 816.8431 + 0.8279x - 0.00002248x^2$; $R^2 = 0.9570$; $p = 0.0006$). In experiment 1, the effect of *Mi* population density on NGR was only significant in cultivar TMG 44B2RF (Fig. 5), with best fit of data by the quadratic model ($y = 15.7147 + 0.0396x - 0.00000098x^2$; $R^2 = 0.9937$; $p = 0.0002$). In contrast, *Me* population density had significant effects on NGR in both cultivars, with a linear fit for IMA 5801B2RF ($y = 31.2759 + 0.1408x$; $R^2 = 0.9053$; $p = 0.0001$), and a quadratic fit for TMG 44B2RF ($y = -5.5307 + 0.0558x - 0.00000158x^2$; $R^2 = 0.9934$; $p = 0.0030$). In experiment 2, the effect of *Mi* population density on NGR was significant in both cultivars and fit the linear model, with a much higher angular coefficient in TMG 44B2RF (Fig. 6). The

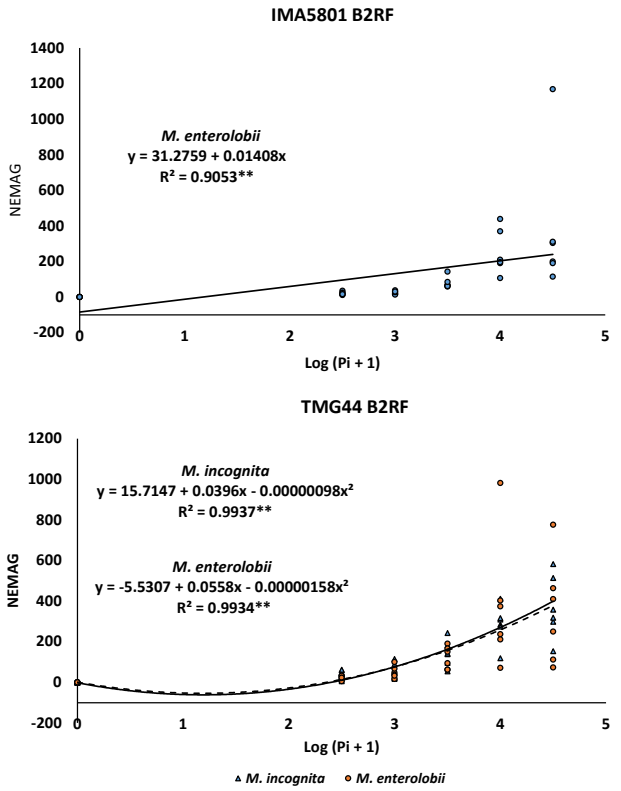

Figure 5. Number of nematodes per gram of root (NGR) (eggs and J_2) in cotton cultivars IMA 5801B2RF and TMG 44B2RF with increasing *Meloidogyne incognita* (solid line) or *Meloidogyne enterolobii* (dashed line) population densities (Pi), in Dourados (experiment 1).

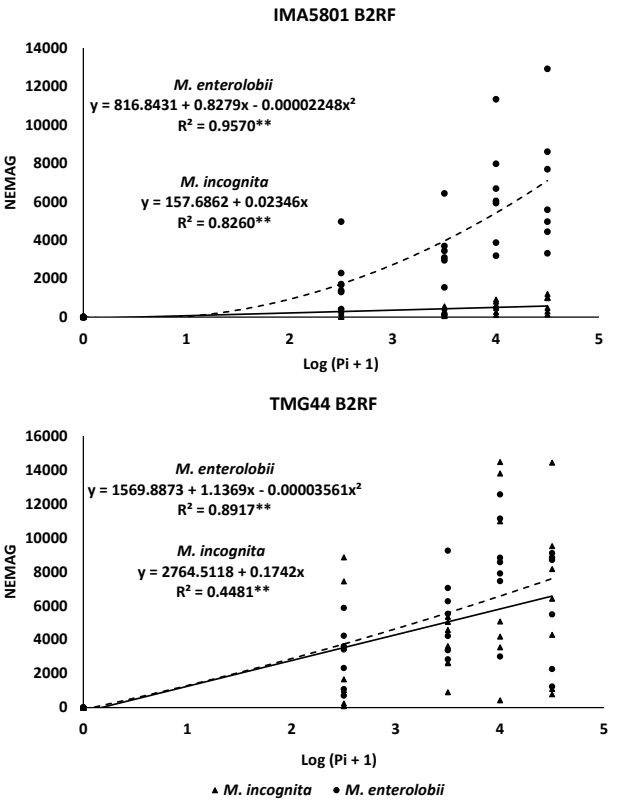

Figure 6. Number of nematodes per gram of root (NGR) (eggs and J_2) in cotton cultivars IMA 5801B2RF and TMG 44B2RF with increasing *Meloidogyne incognita* (solid line) or *Meloidogyne enterolobii* (dashed line) population densities (Pi), in Primavera do Leste (experiment 2).

NGR was similar when the cultivars were inoculated with *Me* but was greater in IMA 5801B2RF at higher population densities.

Significant differences were observed in RF between cultivars, *Meloidogyne* species, and population densities in both experiments. In experiment 1, there was a significant interaction between cultivars and species and between cultivars and population density. In experiment 2, there was a significant interaction between all factors (Tables 1 and 2). With respect to the mean of the two *Meloidogyne* species, RF was higher in cultivar TMG 44B2RF in both experiments (Table 5). Considering the means of both cultivars, experiment 2 exhibited higher RF in plants inoculated with Me; however, cultivar TMG 44B2RF showed no significant RF difference between the two species.

In experiment 1, *Mi* population densities significantly influenced the RF of cultivar TMG 44B2RF, fitting the third-order polynomial model, that is, showing increased RF to a certain population level, with a subsequent decline (Fig. 7). In contrast, the response of RF to inoculation with increasing Me population densities differed between cultivars, with TMG 44B2RF fitting the third-order polynomial model and IMA 5801B2RF fitting the linear model, but with low RF values. In experiment 2, although significant, the effect of Mi population densities on RF was lower in cultivar IMA 5801B2RF than cultivar TMG 44B2RF (Fig. 8). When inoculated with Me, cultivar IMA 5801B2RF showed RF values that fit the third-order polynomial model ($y = 0.9081 + 0.0004x - 0.00000005x^2 + 1.4583E\text{-}12x^3$; $R^2 = 0.2038$; $p = 0.0133$), and cultivar TMG 44B2RF showed RF values that fit the quadratic model ($y = 123.24701 - 0.0160x + 0.00000043x^2$; $R^2 = 0.2513$; $p = 0.0218$).

Both cultivars exhibited lower *Meloidogyne* species multiplication rates in experiment 1 than experiment 2. This might have occurred because experiment 1 was initiated during the winter and, even in the greenhouse, minimum temperatures during the experimental period were abnormally low in 2022, with 49 d of minimum temperatures below 15 °C, considered restrictive to *Mi* (Carter, 1975; Dávila-Negrón and Dickson, 2013) and Me (Veloso et al., 2022) reproduc-

Table 5. Reproduction factor[z] (RF) of *Meloidogyne incognita* (Mi) or *Meloidogyne enterolobii* (Me) in roots of cultivars IMA 5801B2RF and TMG 44B2RF, in Dourados (experiment 1) and Primavera do Leste (experiment 2).

	Experiment 1			Experiment 2		
	Mi	*Me*	Mean	*Mi*	*Me*	Mean
IMA 5801B2RF	0.04 bB[y]	1.15 aA	0.41 a	3.42 bB	32.01 bA	17.8 a
TMG 44B2RF	1.83 aA	0.79 bB	1.49 b	63.34 aA	68.21 aA	65.8 b
Mean	0.93 A	0.97 A		33.4 A	50.2 B	

[z]Original means. For statistical analysis, the original data were transformed into log (x+1).

[y]Means followed by the same lowercase letter in columns and uppercase letter in rows do not differ by the F-test (5%).

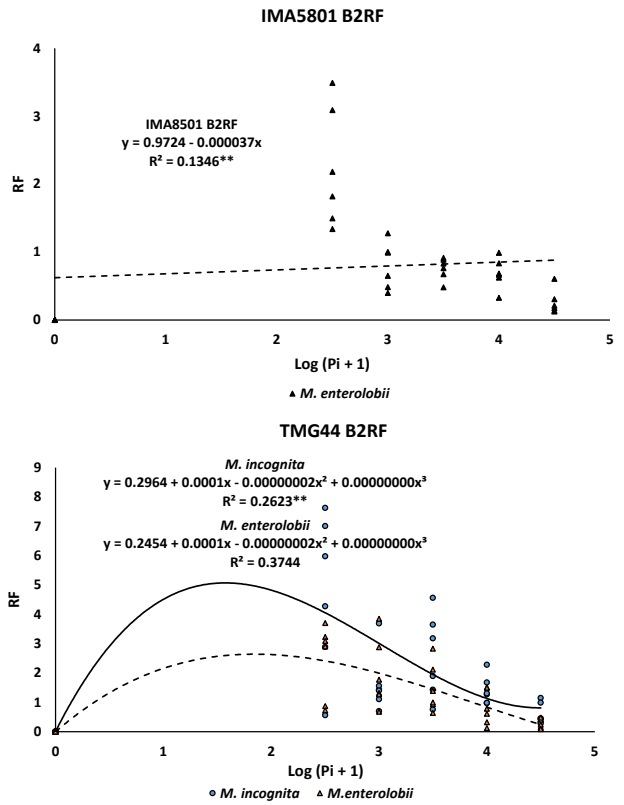

Figure 7. Reproduction factor (RF) of nematodes *Meloidogyne incognita* (solid line) or *Meloidogyne enterolobii* (dashed line) in cotton cultivars IMA5801 B2RF and TMG44 B2RF with increasing population densities (Pi), in Dourados (experiment 1).

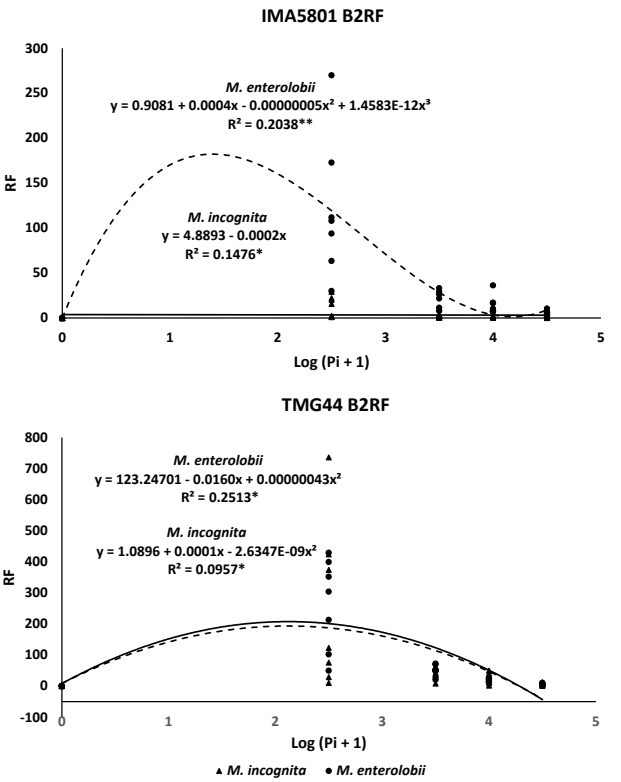

Figure 8. Reproduction factor (RF) of nematodes *Meloidogyne incognita* (solid line) or *Meloidogyne enterolobii* (dashed line) in cotton cultivars IMA5801 B2RF and TMG44 B2RF with increasing population densities (Pi), in Primavera do Leste (experiment 2)).

tion. Although these temperatures limit reproduction, gall formation on the roots was evident and damage could be assessed.

M. enterolobii is an emerging nematode in several crops of economic interest in Brazil. The species severely damaged guava crops in the early 2000s, being subsequently recorded in several other crops (Castro, 2019). Recent records show *Me* parasitizing cotton plants carrying resistance genes to *Mi* in commercial production areas. The objective of this study was to assess the reproductive capacity and ability to cause damage by *Mi* and *Me* at different population densities on two cotton cultivars.

The cotton cultivar IMA 5801B2RF has oligogenic resistance to *Mi*, with QTL resistance on chromosomes 11 (QTL - qMi-C11) and 14 (QTL - qMi-C14) (Belot et al., 2020), which was evidenced by low GI, NGR, and RF, even when subjected to high population densities. However, when inoculated with *Me*, this cultivar behaves similarly to the susceptible cultivar TMG 44B2RF. These results demonstrate that QTL of the genes qMi-C11 and qMi-C14 originating from the

resistance source Auburn 623 RNR (Gutiérrez et al., 2010) do not confer resistance to *Me* in cotton crops, corroborating the results of Galbieri et al. (2020) and Gaudin et al. (2022). Gaudin et al. (2022) clearly demonstrated that these resistance QTLs were ineffective against *Me* in cotton by assessing two different population densities. Similar behavior has already been observed in other crops. Carneiro et al. (2006a) reported damage caused by *Me* in tomato cultivars Débora and Andrea, both carrying the *Mi* gene, which confers resistance to *Mi*, *M. arenaria* (Neal), and *M. javanica* (Gilbert and McGuirre, 1956), and in the silver bell pepper hybrid rootstock, which is resistant to *Mi* and *M. javanica* races 1, 2, 3, and 4 (Braga et al., 2004).

The greater pathogenicity of *Me* compared to *Mi* was evidenced by the higher rate of root galls in both cotton cultivars when inoculated with *Me*. *M. enterolobii* parasitism in cultivar IMA 5801B2RF reduced plant height and SDM, and these responses were influenced by population density. The *Meloidogyne* species had no effect on leaf chlorophyll content and photosynthetic rate.

The influence of nematode infection on leaf chlorophyll content and photosynthesis was observed in some pathosystems involving *Meloidogyne* species (Asmus and Ferraz, 2001; Loveys and Bird, 1973; Melakeberhan et al., 1987). In a pepper (*Capsicum annuum* L.) pathosystem with Me, susceptible cultivars inoculated with 2,000 eggs showed lower chlorophyll contents than non-inoculated plants (Carrillo-Fazio et al., 2020). Specifically in the case of cotton, Lu et al. (2014) inoculated plants of cultivar FM 960BR, susceptible to *Mi*, with 6,000 or 20,000 nematodes in five experiments and, reported lower leaf chlorophyll content in the inoculated plants and inconclusive results on photosynthetic rate reduction, because this rate was reduced in inoculated plants only in two of the five experiments. Our data showed that *Mi* and *Me* parasitism does not interfere with these variables in cotton, even when inoculated with 27,000 eggs/plant. It is noteworthy that Lu et al. (2014) assessed their plants between 75 and 80 DAI, and we assessed them at 57 DAI. Thus, these differences could be related to the phenological stage at which the plants were analyzed. Due to the high demand for photoassimilates in plants at the reproductive stage, the effects of parasitism might be more intense at this stage.

The effect of increasing *Me* population densities on the GI and reduced SDM production were demonstrated in *Solanum quitoense* Lam. crops by Crozzoli et al. (2012). Our data show that the GI increased significantly in cotton plant roots parasitized by *Me* with increasing population density, regardless of the cultivar. Therefore, in the absence of resistant cultivars, it is essential to use management practices that keep the nematode population low in infested areas. Growing soybean cultivars resistant to *Me* in infested areas would be an important management strategy, considering that approximately 85% of cotton in Brazil is grown after soybean. However, there are few resistant soybean cultivars available (Correia, 2023; Dias et al., 2010b; Verssiani et al., 2023). Crop rotation with maize is also an interesting practice, especially considering the large number of cultivars resistant or moderately resistant to *Me* (Dias et al., 2010a). A management alternative is to grow cover crops, including millet, sorghum, and Sudan grass, all resistant to the nematode (Khanal and Harshman, 2022).

Mean NGR and RF were higher with *Me* than *Mi* in both cultivars. However, cultivar IMA 5801B2RF is resistant to *Mi* but susceptible to *Me*, which contributed to this result. The high *Me* multiplication in both cultivars in experiment 2 indicates rapid establishment and population growth in areas where this nematode is introduced, which can be worsened by the ability of *Meloidogyne* species to survive in agricultural soils under low-humidity conditions, common in the cotton-growing region of Brazil, even without favorable hosts (Star, 1993).

In summary, these results show that *Me* is more aggressive than *Mi*, presenting a greater capacity to multiply in cotton crops and therefore can be considered a potential threat to Brazilian cotton farming. In addition, genetic resistance to *Mi* is ineffective for *Me* in cotton plants. To date (2024), there are no known sources of resistance to this nematode in cotton. This means living with this nematode for years or decades without the possibility of managing it through genetic resistance. Efforts must be made to prevent the spread and establishment of high populations of the nematode in cotton production areas using cultural, biological, and chemical management and to find sources of resistance to this nematode in cotton plants.

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