

## Selection of resistant upland cotton genotypes challenged with aggressive isolates of *Meloidogyne incognita* race 3

E. Pires<sup>1</sup>, G.P. Domiciano<sup>2</sup>, R.L. Portz<sup>4</sup>, E.A. Lima<sup>3</sup>, J.L. Belot<sup>5</sup> and C. Furlanetto<sup>3,\*</sup>

<sup>1</sup>Centro Universitário Fundação Assis Gurgacz, Avenida Ministro Cirne Lima, 2565, Jardim Coopagro, 85.903-590, Toledo, Paraná, Brasil

<sup>2</sup>Centro Nacional de Recursos Genéticos e Biotecnologia, Parque Estação Biológica, PqEB, Av. W5 Norte, Brasília, Distrito Federal, 70770-917, Brasil

<sup>3</sup>Universidade de Brasília, Instituto de Ciências Biológicas, Departamento de Fitopatologia, Campus Darcy Ribeiro, Asa Norte, 70.910-900, Brasília, Distrito Federal, Brasil

<sup>4</sup>Universidade Federal do Paraná, R. Pioneiro 2153, Jardim Dallas, 85950-000, Palotina, Paraná, Brasil

<sup>5</sup>Instituto Matogrossense do Algodão, Departamento de Fitopatologia, Estrada Br 070, S/N Zona Rural, 78.850-000, Primavera do Leste, Mato Grosso, Brasil

\*Correspondence: cfurla@hotmail.com

Received: July 11<sup>th</sup>, 2022; Accepted: August 19<sup>th</sup>, 2022; Published: August 25<sup>th</sup>, 2022

**Abstract.** This study aimed to select populations of *M. incognita* race 3 for screening cotton genotypes as part of a breeding program for the development of resistant cotton cultivars. Five isolates of *M. incognita* race 3, collected in Western Paraná, Brazil, were tested for virulence and aggressiveness against the cotton cultivars FM966 (susceptible), IAC 24 (resistant), CD 409, and FMT 701 (moderately resistant) under greenhouse conditions, and following a factorial design with five replicates. Thirty-one cotton genotypes were screened against the three most aggressive isolates of *M. incognita* race 3 tested before and kept under greenhouse conditions following a factorial design with five replicates. Experiments run under greenhouse conditions had single cotton plants inoculated with 5,000 eggs/J2 of *M. incognita* and were assessed at 120 days after inoculation considering the variables gall index, egg mass index, total eggs, and reproduction factor. The same genotypes tested under greenhouse conditions were also grown in a field infested with *M. incognita* race 3 in a randomized block design with 10 replicates. In the field, the *M. incognita* population was monitored by the quantification of J2 forms in soil samples collected before sowing, 60 days after sowing (DAS), and 120 DAS. A gall index score was used to evaluate the roots of cotton genotypes at 120 DAS. The isolate from Umuarama was the most aggressive, followed by Moreira Sales and Iporã. The genotypes CD 05-419, CD 05-945, CD 05-1087, and CD 05-1170 showed resistance against *M. incognita* race 3 under greenhouse and field conditions.

**Keywords:** aggressiveness, genetic resistance, *Gossypium hirsutum*, root-knot nematode, virulence.

## INTRODUCTION

Herbaceous cotton (*Gossypium hirsutum* L.) is one of the most important annual crops in Brazil, due to its excellent economic return and market competitiveness (Dohlman et al., 2019). In the 2020/2021 harvest, Brazil was ranked as the second largest exporter and fourth largest cotton producer. Cotton growing areas in Brazil are spread mainly in the states of Mato Grosso and Bahia, which together respond for nearly 90 percent of all the Brazilian cotton production (Coêlho, 2021; Meyer & Dew, 2022). The high yield obtained in the cotton crop in Brazil has been affected by plant-parasitic nematodes with no cost estimation (Pires et al., 2008; Machado, 2014). In the USA, yield losses caused by *M. incognita* in cotton were estimated at 2,3% in season 2021 (Langstone, 2022).

One of the major nematode species of the cotton crop is the southern root-knot nematode *Meloidogyne incognita* (Lu et al., 2014). This nematode is of great importance in cotton-growing areas worldwide due to its aggressiveness and large host range. The main symptoms caused by this nematode in cotton plants are the presence of galls on the roots and plant growth suppression, which lead to a decrease in crop yield. Cotton losses are mainly caused by the population density of the nematode in the soil and its distribution in the growing area (Starr & Page, 1990).

*M. incognita* complex presents four host races, but only races 3 and 4 are known as parasites of cotton worldwide (Starr & Page, 1990). However, race 3 is prevalent in cotton-growing fields in Brazil (Kirkpatrick & Sasser, 1983; Pires et al., 2008).

The difficulties found when strategies are applied for the control of *M. incognita* in infested fields lie in the large land extension and improper management. The latter factor is the most significant as farmers fail to rotate crops, seeking an immediate return from the land. This leads to a sharp increase in the density of the nematodes, which soon become epidemic (Starr et al., 2007).

In cotton growing areas, chemical control of *M. incognita* may be performed through the use of nematicides. Nonetheless, these molecules are highly toxic and harmful to the environment, and also increase the costs of cotton production (Overstreet et al., 2014). Furthermore, prolonged use of chemical molecules can foster the selection of resistant variants in the nematode population. In addition, the large range of host plants makes crop rotation a difficult task when *M. incognita* is the target.

The use of resistant cotton cultivars is the most desirable method for controlling *M. incognita* for being safe and without additional cost for farmers. Moreover, when growing resistant cotton, the density of *M. incognita* in the soil is decreased (Wheeler et al., 2020), which prevents the appearance of new pathogen biotypes (Starr & Roberts, 2004). The genetic variability present in plant germplasm banks has been investigated by breeders worldwide for incorporating resistance genes in major crops against plant-parasitic nematodes and other plant pathogens (Razukas et al., 2009; Alves et al., 2017).

Different strategies have been used for assessing resistance to *M. incognita* in cotton germplasm collections. Davis & May (2003) and Carneiro et al. (2005) reported the use of single *M. incognita* isolates to assess cotton genotypes without a previous test for aggressiveness. Ogallo et al. (1997) screened cotton germplasm for resistance against

virulent isolates of *M. incognita* with increased reproduction on resistant cotton. However, this procedure may complicate the selection of plant genotypes expressing oligogenic resistance (Zhou et al., 2000) and the selection of other plant genotypes with a different resistant gene (Phillips & Blok, 2008).

In this study, three isolates of *M. incognita* race 3 were selected and used to test the reaction of cotton genotypes, searching for sources of resistance to this nematode.

## MATERIALS AND METHODS

### Collection, establishment, and identification of *M. incognita* isolates

The five isolates of *Meloidogyne incognita* (EST II, race 3) used in this study were previously collected from commercial cotton planting areas in the state of Paraná, Brazil, and kept in a glass-enclosed greenhouse at 25 °C. *Meloidogyne incognita* isolates were identified by the esterase phenotype (Esbenshade & Triantaphyllou, 1990) and host race was determined (Pires et al., 2008), according to Hartman & Sasser (1985).

Single egg masses were extracted from cotton roots and inoculated on tomato plants cultivar Rutgers for nematode reproduction. Tomato plants were grown in pots with 1.5 kg of a sterile substrate (1:1 v/v soil and sand), watered daily, and kept in a greenhouse under temperatures ranging from 25 to 28 °C.

### Extraction of eggs and J2 of *M. incognita* from tomato roots

Sixty days after inoculation (DAI), tomato roots were separated from the shoot, washed with tap water, sectioned into small pieces, and crushed in a blender with sodium hypochlorite (NaOCl) at 0.5% for 60 seconds and under low rotation (Hussey & Barker, 1973). The suspension was passed through a sieve with a pore size of 230 µm stacked over a sieve with a pore size of 25 µm. Eggs and J2 (second-stage juveniles) retained on the sieve with a pore space of 25 µm were transferred to a beaker and quantified on a Peters' slide before inoculation.

### Selection of *M. incognita* isolates

The experiment was carried out in a 5×4 factorial design consisting of the 5 isolates of *M. incognita* race 3 and four cotton varieties: resistant - IAC 24 (Cia et al., 2002), susceptible - FM966 (Galbieri et al., 2009), and moderately resistant - CD 409 and FMT 701 (Galbieri et al., 2009). Cotton plants were inoculated with 5,000 eggs of *M. incognita*, kept in a glasshouse at 25–28 °C, and assessed at 120 DAI based on the following variables: gall index, the total number of eggs, and reproduction factor. This experiment was performed twice in time.

### Cotton genotypes tested in greenhouse and field experiments

Cotton genotypes, developed by the Central Cooperative of Agricultural Research (Coodetec) in collaboration with CIRAD-France, were tested against three populations of *M. incognita* race 3 (Table 1). The cultivars IAC 24 (Cia et al., 2002) and FM966 (Galbieri et al., 2009) were used as resistant and susceptible cotton varieties, respectively. The cultivars FMT 701, and CD 409, which are moderately resistant to *M. incognita* (Galbieri et al., 2009), were also included in this work.

**Table 1.** Genealogy of cotton genotypes and cultivars challenged with *Meloidogyne incognita* race 3

Genotype/Cultivar	Crossings	Genotype	Crossings
CD 406	<sup>1</sup> OC165 x Sicala V1	CD05-206	<sup>12</sup> CD98-39 x CD98-378
CD 408	<sup>1</sup> OC165 x Sicala V1	CD05-243	<sup>12</sup> CD98-39 x CD98-378
CD 409	<sup>1</sup> OC92-165 x Sicala 3-2	CD05-419	<sup>13</sup> CD991 x CD97-545
CD 410	<sup>2</sup> DPAc90 x P288	CD05-485	<sup>14</sup> CD97-122 x CD96-252
CD02-621	<sup>3</sup> OC92-165 x SP 8324	CD05-700	N320-2-9 x CD405
CD02-1637	<sup>1</sup> OC92-165 x Sicala 3-2	CD05-865	*N419-1-191 x CD401
CD03-5198	<sup>4</sup> SP8334 x Ston BR110	CD05-945	N315 RNR x CD401
CD04-4939	<sup>5</sup> CD98-218 x OC94-434	CD05-1039	<sup>15</sup> M315 RNK x OC96-276(CD404)
CD04-3361	<sup>6</sup> CD98-213 x CD98-420	CD05-1087	<sup>16</sup> M155 RKN x CD 401
CD04-3040	<sup>7</sup> CD98-578 x CD98-378	CD05-1170	<sup>16</sup> M155 RKN x CD 401
CD04-3278	<sup>8</sup> CD98-39 x CD98-578	CD05-1222	<sup>17</sup> M155 RKN x CD 405
CD04-3816	<sup>9</sup> CD98-991 x CD97-122	CD05-1323	<sup>18</sup> M155 RKN x OC94-434
CD04-4721	<sup>10</sup> CD98-218 x CD405	CD04-5281	<sup>19</sup> CD98-450 x OC94-434
CD04-5081	<sup>11</sup> CD98-361 x CD 401	CD04-2990	<sup>7</sup> CD98-578 x CD98-378

<sup>1</sup>(P288/DP41)/Unknown; <sup>2</sup>Unknown/(Allen x HAR); <sup>3</sup>(P288/DP41)/Fundo US; <sup>4</sup>Fundo Argentino; <sup>5</sup>92-165x SicalaV1/AllenxHAR/IAC20; <sup>6</sup>92-165xSicala32/HAR/IAC20; <sup>7</sup>(Yuc/TniHoa/Au56)/HR102/DPAc90/Auburn56; <sup>8</sup>P288/DP41/(Yuc/TniHoa/Au56)/HR102; <sup>9</sup>SP8334 x DPAc90/IRCT223 x P288; <sup>10</sup>92-165 x SicalaV1/(CNPA86-387xP288)xPR3060/87; <sup>11</sup>Sealand542xIAC20Reba/CD401; <sup>12</sup>92-165xSicala32/DPAc90/Auburn56; <sup>13</sup>SP8334x DPAc90/Sealand542xIAC20; <sup>14</sup>IRCT223xP288; <sup>15</sup>Auburn634/DeltaPine61/SP8334/DPAc90; <sup>16</sup>Auburn634/Coker310/CD401; <sup>17</sup>Auburn634/Coker310/N'Kourala/(Allen x HAR)/Auburn56; <sup>18</sup>Auburn634/Coker310/HAR/IAC20; <sup>19</sup>Sealand542 X IAC20Reba/HAR/IAC20.

### Inoculation of *M. incognita* race 3 on cotton genotypes

Cotton genotypes were kept in a greenhouse at a temperature of 27 °C and 60% relative humidity. Single cotton plants were grown in plastic tubes of 7×18 cm containing sterile substrate composed of soil and sand in the ratio of 1.5:1 and fertilized with 2 grams of N-P-K 8-20-20.

Plants with two true leaves were inoculated with 3 mL of a suspension containing 5,000 eggs and juveniles of *M. incognita* race 3. Cotton plants were evaluated at 120 DAI based on the following variables: Number of galls (GA), gall index (GI) (Taylor & Sasser, 1978), total eggs (Coolen & D'Herde, 1972), and reproduction factor (RF) (Oostenbrink, 1966). For egg extraction, cotton roots were ground in a blender in a solution of 0.5% sodium hypochlorite.

The quantification of eggs and juveniles was determined on a Peters' slide using a light microscope. The cotton plants were watered twice a day during the experiment. The experimental design was completely randomized with 31 treatments (cotton genotypes/varieties) and ten replicates.

Data obtained for eggs were transformed to log X + 1. The RF was calculated using the equation  $RF = Fp/Ip$ , where Fp means final population and Ip means initial population. Cotton genotypes with RF values < 1.0 were classified as resistant (R), moderately resistant (MR) when  $1 \geq RF < 2$ , moderately susceptible (MS) when  $2 \geq RF < 3$ , and susceptible (S) when  $RF > 3$ . This classification followed Khan et al. (2016).

### Field experiment

Cotton genotypes were grown in a commercial area of 120.75 m<sup>2</sup> infested with *M. incognita* race 3 where cotton had been cultivated for three years without rotation. The area is located in the municipality of Moreira Sales, northwest of Paraná state, Brazil.

A randomized block experimental design was adopted with 31 treatments (cotton genotypes/cultivars) and 10 replicates. Seeds of each treatment (Table 3) were sown with 50 cm plant spacing and row spacing. The cultivars IAC-24 and FM 966 were used as resistant and susceptible checks, respectively.

The population of *M. incognita* found in Moreira Sales was also collected and tested in the greenhouse experiments. Analysis of variance was used on the data, and the means of the treatments were compared by the Scott-Knott clustering algorithm at a 5% significance level (Scott & Knott, 1974). All analyses were performed using the SISVAR software (Ferreira, 2011).

### Quantification of J2 in soil samples and physical and chemical soil analyses

Soil samples were collected for chemical, physical, and nematological analyses. A composite sample of ten cores and 1 kg of soil was selected at random in the experimental area. The grid had one composite sample composed of ten cores. Soil chemical contents were used for fertilizer recommendation. For quantification of J2, soil samples were collected at 0–20 cm depth before sowing and after sowing at 60 days and 120 days.

### Extraction of nematodes

Nematodes were extracted from 100 cm<sup>3</sup> of soil following Jenkins (1964), and eggs were extracted from cotton roots according to Coolen & D’Herde (1972). A sieve with a pore size of 300 µm was stacked over a sieve with a pore size of 37 µm for the separation of nematodes. Eggs and J2 of *M. incognita* were quantified under a light microscope using a Peters’ slide.

### Evaluation of the field experiment

For the field trial, an assessment was conducted 126 days after sowing. Cotton plants were uprooted and the root system was evaluated for root gall rating on a scale of 0 (none) to 5 (severe), following Colyer et al. (2000). Each interval in the rating scale was related to its correspondent resistance level, according to Khan et al. (2016) (Table 2).

**Table 2.** Rating-scale for galls to assess the resistance of cotton genotypes to *M. incognita* race 3 in the field experiment (Khan et al., 2016)

Score	Galls	Ranking
0	0	HR
1	0–3	R
2	4–10	MR
3	11–30	MS
4	31–100	S
5	> 100	HS

## RESULTS AND DISCUSSION

The isolate from Umuarama (UM) was the most virulent and aggressive for most cotton varieties, considering the variables number of galls (GA), Gall index (GI), total eggs, and reproduction factor, followed by the isolates from Moreira Sales (MS), Iporã (IP), Pérola (PL) and Santa Lúcia (SL). Regarding the variables GA and GI, the UM isolate was more aggressive than PL and SL isolates but it did not differ from the isolates MS and IP. MS and IP isolates were more aggressive than PL and SL isolates for most variables, except for the variable GI (Table 3). Based on the results, UM, MS, and IP were selected for screening the cotton genotypes used in this study.

**Table 3.** Virulence and aggressiveness of *Meloidogyne incognita* race 3 isolates on cotton cultivars for germplasm assessment

Cultivar	Variable																							
	Ga (values x 10)						Gi						Eggs (valuesx10.000)						Rf					
Isolate	Um	Ms	Ip	Pl	Sl	V	Um	Ms	Ip	Pl	Sl	V	Um	Ms	Ip	Pl	Sl	V	Um	Ms	Ip	Pl	Sl	V
FM 966 <sup>1</sup>	27	14	13	7.3	6.2	135 C	5	5	5	3.8	4	4.6 C	5	3.2	2.1	1.1	0.8	2.4	1	6.4	4.7	1.8	1.7	6.1 A
IAC-24 <sup>2</sup>	2.9	1.9	1.5	1.1	1	17 A	2.8	2.6	2.4	2.0	1.8	2.3 A	1.3	0.8	0.5	0.5	0.3	0.6	2.5	1.6	0.9	0.7	0.5	1.5 C
CD 409 <sup>3</sup>	16	12	8.4	4.1	6	92 B	5	4.6	4	3.2	3.8	4.1 C	2.1	1.4	1.7	0.7	0.6	1.3	4.3	3.5	2.7	1.3	1.1	3.2 B
FMT 701 <sup>3</sup>	16	7.5	7.6	3.1	3.1	74 B	5	3.8	3	3.5	3.4	3.7 B	2.9	1.6	0.9	0.5	0.5	1.3	5.9	3.2	1.8	1	1.2	3.3 B
Isolate	15 a	8.7 b	12 B	3.9 c	4.1 c		4.4 a	4 ab	2.9 Ab	3.1 bc	3.2 bc		0.3 a	0.2 b	1.3 c	0.7 d	0.5 d		5.6 a	3.7 b	2.5 c	1.2 d	1.1 d	
CV%	18.27						6.12						15.92						12.60					

Data transformed to square root of X + 1 with original data kept; mean values represent five plants/treatment and a total of 99 degrees of freedom; averages followed by the same letter in the column (varieties) or row (isolates) did not differ by Tukey' test at 5% probability; Ga = number of galls; Gi = Gall index (Taylor & Sasser, 1978): 0 = no galls or egg masses; 1 = 1-2; 2 = 3-10; 3 = 11-30; 4 = 31-100, and 5 >100; Rf = Reproduction factor; Pl = Population from Pérola, PR; Ip = Population from Iporã, PR; Um = Population from Umuarama, PR; Ms = Population from Moreira Sales, PR; Sl = population from Santa Lúcia, PR;

<sup>1</sup>susceptible cultivar - Fibermax 966; <sup>2</sup>resistant cultivar; <sup>3</sup>moderately resistant cultivars. V = varieties. CV = Coefficient of variation; Combined analysis of greenhouse experiments 1 and 2.

According to Castagnone-Sereno (2002), the genetic variability of plant-parasitic nematodes should be taken into account when evaluating plant resistance and its durability. Additionally, previous tests with a large collection of nematode isolates should reduce the risks of a rapid breakdown of plant resistance genes.

Differences in the reproduction of *M. incognita* isolates in cotton genotypes were also reported by Silva et al. (2014) and for other root-knot nematodes by Van der Beek et al. (1998) on potato cultivars. This latter, reports an isolate-by-cultivar interaction between *M. hapla* and potato cultivars.

The gall and RF values obtained for the cotton genotype treatments after 120 DAI with the three isolates allowed the genotypes to be ranked into different categories based on their reaction to *M. incognita* (Table 4).

**Table 4.** Mean of galls and reproduction factor of three isolates of *Meloidogyne incognita* race 3 inoculated on cotton genotypes and cultivars under greenhouse experiments

Treatment	Galls	Reproduction factor
FMT 701	217.40 e	2.08 c
CD 406	225.20 e	2.13 c
CD 408	295.53 g	3.00 d
CD 409	220.33 e	2.04 c
CD 410	272.06 f	2.58 d
CD02-621	157.13 d	1.42 b
CD02-1637	190.86 d	1.73 c
CD03-5198	349.53 g	4.21 f
CD04-4939	326.13 g	3.54 e
CD04-3361	326.60 g	3.79 e
CD04-3040	236.06 e	2.39 c
CD04-3278	263.06 f	2.83 d
CD04-3816	150.26 d	1.27 b
CD04-4721	189.00 d	2.27 c
CD04-5081	173.86 d	1.41 b
CD04-5281	225.53 e	1.74 c
CD05-206	310.40 g	2.67 d
CD05-243	514.60 h	4.60 f
CD05-419	113.26 c	0.81 a
CD05-485	109.40 c	0.81 a
CD05-700	207.73 d	1.18 b
CD05-865	350.86 g	1.89 c
CD05-945	74.60 b	0.56 a
CD05-1039	261.80 f	2.14 c
CD05-1087	46.70 a	0.37 a
CD05-1170	59.00 a	0.47 a
CD05-1222	56.40 a	0.48 a
CD05-1323	61.13 a	0.54 a
CD04-2990	165.13 d	2.00 c
FM 966	615.73 i	16.53 g
IAC 24	84.00 b	1.03 b
CV (%)	14.37	13.37

Also, the isolate UM was more aggressive than MS and IP, while MS was more aggressive than IP isolate in the greenhouse experiments, as shown in Table 5.

**Table 5.** Overall mean of galls and reproduction factor of three isolates of *Meloidogyne incognita* race 3 on cotton cultivars in greenhouse experiments

Isolate	Galls	Reproduction Factor
IP	147.17 a	1.49 a
MS	195.70 b	1.69 b
UM	319.96 c	2.02 c

\*Means followed by the same letter do not differ by Tukey's test at 5% probability. Data transformed by the *square root of X + 1* with original data kept; combined analysis of greenhouse experiments 1 and 2. IP = Iporá, MS = Moreira Sales, UM = Umuarama.

In both greenhouse experiments, the genotypes CD05-1087, CD05-1170, CD05-1222, and CD05-1323 produced the lowest values for GA (averages ranging from 47 to 61) and RF (averages ranging from 0.4 to 0.5). CD05-945, CD05-419, and CD05-485 produced more galls on the roots (averages ranging from 109 to 113) than the previously cited genotypes, but their RFs did not differ statistically (average of 0.81).

The cultivar IAC 24, used as a resistance check, had a GA mean of 84.0 and RF mean of 1.0. The RF of IAC-24 did

not differ statistically from the RFs of CD05-700 (mean of 1.18), CD04-5081 (mean of 1.41), CD04-3816 (mean of 1.27), and CD02-621 (mean of 1.42), but these genotypes had a larger number of galls on the roots, which ranged from 150 to 173.

The genotypes FMT 701, CD 406, CD 409, CD04-3040, and CD04-5281 produced more galls (217 to 225) on the roots than CD02-1637 and CD04-4721 (189 to 191), but these genotypes did not differ statistically from each other based on the RFs, which ranged from 1.7 to 2.4.

Genotypes with means of the RF ranging from 2.6 to 3.0 are statistically similar, but for the GA variable, CD04-3278 (263 galls) and CD 410 (272 galls) are statistically different from CD 408 (295 galls) and CD 05-206 (310 galls).

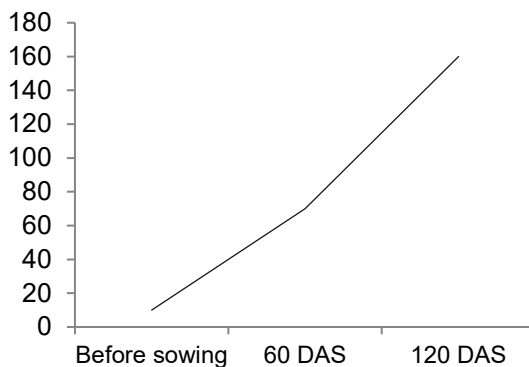
Other genotypes, such as CD04-4939 (mean RF of 3.5, 326 galls) and CD04-3361 (RF of 3.8, 327 galls) are statistically different from the genotypes CD03-5198 (RF of 4.2, 349 galls), and CD05-243 (RF 4.6, 515 galls). The susceptible check, cultivar FM966, had the highest values for means of RF (16.5) and galls (616).

The UM isolate was the most aggressive, inducing an overall average of 320 galls per cotton plant and RF mean of 2.0, followed by MS with 196 galls and RF of 1.7, and IP with 147 galls and RF of 1.5 (Table 4). There was a positive correlation between the galls produced in different cotton genotypes and the RF of the three isolates tested ( $r = 0.86$ ;  $R^2 = 0.97$ ;  $P < 0.05$ ).

Soil samples analysis revealed that silt, sand, and clay contents were at 4%, 88%, and 8%, respectively. The analysis revealed the presence of J2 of *M. incognita* in the soil of the experimental area before the beginning of the field trial, and an increase in the J2 density during cotton cultivation (Fig. 1).

The results obtained in the field showed that the evaluation by rating scale allowed the separation of the genotypes into 4 groups according to their resistance level (Table 6). According to the evaluation criterion adopted (rating scale), CD05-419 (GI = 1.90), CD05-1087 (GI = 1.70), CD05-1170 (GI = 1.70), and CD05-945 (GI = 1.60) were classified as resistant due to GIs less than 2.0. The other genotypes were classified as moderately resistant, moderately susceptible, and susceptible. The susceptible check, FM 966, had a GI of 4.40 and was classified as susceptible. The resistant check, IAC-24, had a GI of 2.20 and was classified as moderate resistant (Table 6).

Several works addressing the resistance level of cotton genotypes from germplasm collections were previously reported by Shepherd (1974, 1982), Shepherd et al. (1996), and Starr & Smith (1999). Other studies involving the search for new sources of resistance to root-knot nematodes were reported by Sheperd (1983), Mota et al. (2013), and inheritance of resistance in cotton accessions (Faske & Starr, 2009; Alves et al., 2017).



**Figure 1.** Dynamics of *M. incognita* J2 in the soil of a field experiment assessed before sowing and at 60 and 120 days after sowing (DAS).



**Table 6.** Host status of cotton genotypes and cultivars inoculated with *M. incognita* race 3 and assessed under greenhouse and field conditions

Genotype	Host Status*	Field Score	Host Status**	Genotype	Host Status*	Field Score	Host Status**
CD 05-945	R	1.60 a <sup>#</sup>	R	CD 04-5281	MR	2.40 b	MR
CD 05-1087	R	1.70 a	R	CD 409	S	2.60 b	MR
CD 05-1170	R	1.70 a	R	CD 02-621	MR	2.60 b	MR
CD 05-419	R	1.90 a	R	CD 05-700	MS	2.70 b	MR
CD 05-1222	MR	2.00 a	MR	CD 02-1637	MS	2.80 c	MR
CD 05-1323	MR	2.00 a	MR	CD 410	S	2.90 c	MR
CD 05-485	R	2.00 a	MR	FMT 701	MR	3.00 c	MS
CD 04-3816	MR	2.10 a	MR	CD 05-1039	MS	3.10 c	MS
CD 04-4939	MR	2.20 b	MR	CD 408	S	3.20 c	MS
IAC - 24	MR	2.20 b	MR	CD 406	MS	3.60 d	MS
CD 04-3361	MR	2.20 b	MR	CD 03-5198	S	3.70 d	MS
CD 04-3278	MR	2.20 b	MR	CD 05-865	MS	3.70 d	MS
CD 04-3040	MR	2.30 b	MR	CD 05-243	S	3.80 d	MS
CD 04-4721	MR	2.40 b	MR	CD 05- 206	MS	3.90 d	MS
CD 04-5081	MR	2.40 b	MR	Fiber Max 966	S	4.40 e	S

\*Column showing host status of cotton genotypes for *M. incognita* in the greenhouse experiments;

\*\*Column containing the ranking of cotton genotypes for *M. incognita* in the field experiment;

# Means followed by the same letter do not differ by the Scott-Knott test at 5% probability;

\*\*\*Ranking adapted from Khan et al. (2016).

The status of some cotton genotypes was different when comparing data from field and greenhouse. CD05-1222 (RF = 0.48) and CD05-1323 (RF = 0.54) were classified as resistant in the greenhouse experiments and moderate resistant in the field (mean of GI = 2.0). In this case, these genotypes were classified as presenting moderate resistance.

Differences in the response of cotton genotypes to the nematode *M. incognita* were also reported by Ogallo et al. (1997) and Galbieri et al. (2009), for tests carried out under greenhouse and field conditions.

Field experiments are challenging because plants are exposed to different climate and soil conditions and also to biotic stresses caused by organisms other than nematodes. All this contributes to a better understanding of the cotton response to plant parasites such as the root-knot nematode *M. incognita*. In this study, the genotypes CD05-419, CD05-1170, CD05-1087, and CD05-945, were classified as resistant to *M. incognita* due to the performance obtained under greenhouse and field conditions. These resistant cotton genotypes will be targeted for further studies about their agronomic performance aiming at the development of new cultivars.

## CONCLUSIONS

The results obtained in this study allowed the selection of four cotton genotypes (CD05-945, CD05-1170, CD05-1087, and CD05-419), through greenhouse and field experiments, for which three *M. incognita* isolates had a low reproductive rate. Additionally, the selection of virulent and aggressive isolates of *M. incognita* before screening genotypes for resistance was very important and helpful in the selection of resistant cotton genotypes.

ACKNOWLEDGEMENTS. The authors thank Coodetec, a Dow AgroSciences group company for agricultural development, production, and commercialization, the University of Brasília - Brazil, and the State University of Western Paraná - Brazil, by supporting this research.

## REFERENCES

- Alves, G.C.S., Barbosa, V.H.S., Giband, M., Barroso, P.A.V., Rodrigues, F. & Rocha, M.R. 2017. Inheritance of resistance to *Meloidogyne incognita* race 3 in cotton accession TX 25. *Acta Sci. Agron* **39**, 331–337.
- Carneiro, R.M.D.G., Neves, D.I., Falcão, R., Paes, N.S., Cia, E. & Sá, M.F.G. 2005. Resistance of cotton genotypes to *Meloidogyne incognita* race 3: reproduction and histopathology. *Nematol Bras* **29**, 1–10.
- Castagnone-Sereno, P. 2002. Genetic variability of nematodes: a threat to the durability of plant resistance genes? *Euphytica* **124**, 193–199.
- Cia, E., Fuzatto, M.G., Pizzinatto, M.A. & Bortoletto, N. 2002. A scale for classifying cotton disease resistance. *Summa Phytopathol.* **28**, 28–32.
- Coêlho, J.D. 2021. Cotton: production and markets. Caderno Setorial ETENE 166, pp. 1–11.
- Colyer, P.D., Kirkpatrick, T.L., Caldwell, W.D. & Vernon, P.R. 2000. Root-Knot Nematode Reproduction and Root Galling Severity on Related Conventional and Transgenic Cotton Cultivars. *J Cotton Sci* **4**, 232–236.
- Coolen, W.A. & D’Herde, C.J. 1972. *A method for the quantitative extraction of nematodes from plant tissue*. Ghent, Merelbeke, Belgium, State Agricultural Research Centre, 77 pp.
- Davis, R.F. & May, O.L. 2003. Relationships Between Tolerance and Resistance to *Meloidogyne incognita* in Cotton. *J. Nematol.* **35**, 411–416.
- Dohlman, E., Johnson, J., MacDonald, S., Meyer, L. & Soley, G. 2019. The World and United States Cotton Outlook for 2019/2020. Agricultural Outlook Forum, USDA, US. [https://www.usda.gov/sites/default/files/documents/Leslie\\_Meyer.pdf](https://www.usda.gov/sites/default/files/documents/Leslie_Meyer.pdf)
- Esbenshade, P.R. & Triantaphyllou, A.C. 1990. Isozyme phenotypes for the identification of *Meloidogyne* species. *J. Nematol.* **22**, 10–15.
- Faske, T.R. & Starr, J.L. 2009. Mechanism of resistance to *Meloidogyne incognita* in resistant cotton genotypes. *Nematropica* **39**, 281–288.
- Ferreira, D.F. 2011. Sisvar: a computer statistical analysis system. *Ciênc. Agrotec.* (UFPA) **35**, 1039–1042.
- Galbieri, R., Fuzatto, M.G., Cia, E., Lüders, R.R., Machado, A.C.Z. & Boldt, A.F. 2009. Reaction of cotton cultivars to *Meloidogyne incognita* under field and greenhouse conditions in the state of Mato Grosso. *Trop Pl Pathol* **34**, 18–23.
- Hartman, K.M. & Sasser, J.N. 1985. Identification of *Meloidogyne* species on the basis of differential host test and perineal pattern morphology. In Sasser, J.N. & Carter, C.C. (eds): *An Advanced Treatise on Meloidogyne*. North Carolina State University, Raleigh, US, pp. 69–77.
- Hussey, R.S. & Barker, K.R. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. *Plant Dis. Rep.* **57**, 1025–1028.
- Jenkins, W.R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. *Plant Dis Rep* **48**, 692.
- Khan, MA, Khan, SA & Khan, RW. 2016. Evaluation of resistance to root knot nematode (*Meloidogyne incognita*) in cotton cultivars. *Transylv. Rev.* **24**, 3201–3208.
- Kirkpatrick, T.L. & Sasser, J.N. 1983. Parasitic variability of *Meloidogyne incognita* populations on susceptible and resistant cotton. *J. Nematol.* **15**, 302–307.

- Langstone, D. 2022. Cotton disease loss estimate committee report, 2021. Beltwide Cotton Conferences, San Antonio, TX, pp. 219–22.
- Lu, P., Davis, R.F., Kemerait, R.C., van Iersel, M.W. & Scherm, H. 2014. Physiological effects of *Meloidogyne incognita* infection on cotton genotypes with differing levels of resistance in the greenhouse. *J. Nematol.* **46**, 352–359.
- Machado, A.C.Z. 2014. Current nematode threats to Brazilian agriculture. *Curr Agric Sci Technol* **20**, 26–35.
- Meyer, L.A. & Dew, T. 2022. Cotton and Wool outlook: July 2022. USDA, *Economic Research Service*, pp. 1–8.
- Mota, F.C., Alves, G.C.S., Giband, M., Gomes, A.C.M.M., Sousa, F.R., Mattos, V.S., Barbosa, V.H.S., Barroso, P.A.V., Nicolef, M., Peixoto, J.R., Rocha, M.R. & Carneiro, R.M.D.G. 2013. New sources of resistance to *Meloidogyne incognita* race 3 in wild cotton accessions and histological characterization of the defense mechanisms. *Plant Pathol.* **62**, 1173–1183.
- Ogallo, J.L., Goodell, P.B., Eckert, J. & Roberts, P.A. 1997. Evaluation of NemX, a new cultivar of cotton with high resistance to *Meloidogyne incognita*. *J. Nematol.* **29**, 531–7.
- Oostenbrink, M. 1966. *Major characteristics of the relation between nematodes and plants*. Mededelingenvoor Landb Hoogeschool Wageningen, Netherland, **66**, pp. 3–46.
- Overstreet, C., McGawley, E.C., Khalilian, A., Kirkpatrick, T.L., Monfort, W.S., Henderson, W. & Mueller, J.D. 2014. Site specific nematode management-development and success in cotton production in the United States. *J. Nematol.* **46**, 309–320.
- Phillips, M.S. & Blok, V.C. 2008. Selection for reproductive ability in *Globodera pallida* populations in relation to quantitative resistance from *Solanum vernei* and *S. tuberosum* ssp. *Andigena* CPC2802. *Plant Pathol.* **57**, 573–580.
- Phillips, M.S. & Blok, V.C. 2011. Selection for reproductive ability in *Globodera pallida* populations in relation to quantitative resistance from *Solanum vernei* and *S. tuberosum* ssp. *andigena* CPC2802. *Plant Pathol.* **57**, 573–580.
- Pires, E., Santana, H., Nasu, E.G.C., Belot, J.L. & Furlanetto, C. 2008. Ocorrência de *Meloidogyne incognita* raça 3 em lavouras de algodão na região Noroeste do Paraná. *Nematol Bras* **32**, 81–83.
- Razukas, A., Jankauskiene, Z., Jundulas, J. & Asakaviciute, R. 2009. Research of technical crops (potato and flax) genetic resources in Lithuania. *Agron Res.* **7**, 59–72.
- Scott, A.J. & Knott, M. 1974. A cluster analysis method for grouping means in the analysis of variance. *Biometrics* **30**, 507–512.
- Silva, E.H., Mattos, V.S., Furlanetto, C., Giband, M., Barroso, P.A.V., Moita, A.W., Jorge-Junior, A., Correa, V.R., Castagnone-Sereno, P. & Carneiro, R.M.D.G. 2014. Genetic variability and virulence of *Meloidogyne incognita* populations from Brazil to resistant cotton genotypes. *Eur. J. Plant Pathol.* **139**, 195–204.
- Shepherd, R.L. 1974. Registration of ‘Auburn 623 RNR’ cotton germplasm. *Crop Sci* **35**, 373–375.
- Shepherd, R.L. 1982. Registration of three germplasm lines of cotton (Reg. Nos. GP167 to GP174). *Crop Sci.* **22**, 692. 124.
- Shepherd, R.L. 1983. New sources of resistance to root-knot nematodes among primitive cottons. *Crop Sci.* **23**, 999–1001.
- Shepherd, R.L., McCarty, J.C., Jr., Jenkins, J.N. & Parrot, W.L. 1996. Registration of nine cotton germplasm lines resistant to root-knot nematode. *Crop Sci.* **36**, 820.
- Starr, J.L. & Page, S.L.J. 1990. Nematode parasites of cotton and other tropical fiber crops. In Luc, M., Sikora, R.A. & Bridge, J. (eds): *Plant parasitic nematodes in subtropical and tropical agriculture*. CAB International, Oxon, UK, pp. 539–556.

- Starr, J.L. & Smith, C.W. 1999. Registration of three root-knot nematode-resistant germplasms. *Crop Sci.* **39**, 303.
- Starr, J.L., Koenning, S.R., Kirkpatrick, T.L., Robinson, A.F., Roberts, P.A. & Nichols, R.L. 2007. The future of nematode management in cotton. *J. Nematol.* **39**, 283–294.
- Taylor, A.L. & Sasser, J.N. 1978. Biology, identification, and control of root-knot nematodes (*Meloidogyne* species). Raleigh, North Carolina State University, 111 pp.
- Van der Beek, J.G., Vereijken, P.F.G., Poleij, L.M. & Van Silfhout, C.H. 1998. Isolate-by-cultivar interaction in root-knot nematodes *Meloidogyne hapla*, *M. chitwoodi*, and *M. fallax* on potato. *Can. J. Bot.* **76**, 75–82. doi: 10.1139/b97-161
- Wheeler, T.A., Siders, K., Monclova-Santana, C. & Dever, J.K. 2020. The relationship between commercial cotton cultivars with varying *Meloidogyne incognita* resistance genes and yield. *J. Nematol.* **52**, 1–8.
- Zhou, E., Wheeler, T.A. & Starr, J.L. 2000. Root galling and reproduction of *Meloidogyne incognita* isolates from Texas on resistant cotton genotypes. *J. Nematol.* **32**, 513–518.