Damage quantification and reaction of bean genotypes *(Phaseolus vulgaris L.)* to *Meloidogyne incognita* race 3 and *M. javanica*

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ABSTRACT


The damage and the resistance levels of cultivars and accessions of common beans rescued in the South and mountain regions of Espirito Santo State, Brazil, to *M. incognita* race 3 and *M. javanica* parasitism were evaluated under a greenhouse. Four rescued bean genotypes (“FORT-10”, “FORT-13”, “FORT-16” and “FORT-19”) and 2 commercial cultivars: “Pérola”; and “Aporé”, were tested. The cultivar “Rico-23” was included as standard of susceptibility to nematodes and non-inoculated plants constituted the control. Thus, the experiment was carried out in a completely randomized design in 3 treatments considering nematodes x 7 (genotypes and bean cultivars) factorial arrangement, with 7 replicates. Data were measured at 50 days after plant inoculation. For damage quantification, the following variables were evaluated: plant height (PHE), number of nodes (NNO), number of trifoliolate leaves (NRT), fresh matter weight (FWE) and dry matter weight (DWE) of shoots, root weight (RWE), number of root nodules (NRO) and final population (FPO) of nematodes per root system. There were no significant differences between the effects caused by *M. incognita* and *M. javanica*, but both species showed inferior values of PHE, NNO, NRT, RWE, FWE and DWE compared to controls. Concerning the levels of resistance of bean plants to *M. incognita*, the genotypes “FORT-10”, “FORT-13”, “Aporé” and “FORT-16” behaved as moderately resistant, the cultivars “Rico 23” and “Pérola” low resistant, and the genotype “FORT-19” as highly susceptible. When parasitized by *M. javanica*, the beans “FORT-19”, “Rico-23”, “FORT-16” and “FORT-13” were low resistant, “Pérola” and “Aporé” susceptible and “FORT-10” highly susceptible.

Keywords: Growth reduction, resistance, bean, root-knot nematode

RESUMO


Avaliaram-se danos e os níveis de resistência de cultivares e acessos de feijoeiro-comum resgatados nas regiões Sul e Serrana do Estado do Espírito Santo ao parasitismo de *M. incognita* raça 3 e *M. javanica* em casa de vegetação. Foram testados quatro genótipos resgatados de feijoeiro (“FORT-10”, “FORT-13”, “FORT-16” e “FORT-19”), e mais 2 cultivares comerciais: “Pérola”; e “Aporé”. O cultivar “Rico-23” foi incluído como padrão de suscetibilidade aos nematóides e plantas não inoculadas constituíram a testemunha. Desta forma, o experimento foi conduzido em delineamento inteiramente casualizado em esquema fatorial 3 (tratamentos considerando nematóides) x 7 (genótipos e cultivares de feijoeiros), com 7 repetições. Os dados foram mensurados aos 50 dias após a inoculação das plantas. Para a quantificação de danos foram avaliadas: altura das plantas (ALT), número de nós (NOS), número de folhas trifolioladas (NFT), peso da matéria fresca (PMF) e da matéria seca (PMS) da parte aérea, peso de raiz (PR), número de nódulos radiculares (NOD) e população final (PF) de nematóides por sistema radicular. Não foram observadas diferenças significativas entre os efeitos causados por *M. incognita* e *M. javanica*, porém ambas as espécies apresentaram valores inferiores quanto à ALT, NOS, NFT, PR, PMF e PMS em relação às testemunhas. Em relação aos níveis de resistência dos feijoeiros a *M. incognita* os feijoeiros “FORT-10”, “FORT-13”, “Aporé” e “FORT-16” comportaram-se como moderadamente resistente, as cultivares “Rico 23” e “Pérola” como pouco resistente e o genótipo “FORT-19” como altamente suscetível. Quando parasitados por *M. javanica*, os feijoeiros “FORT-19”, “Rico 23”, “FORT-16” e “FORT-13” foram pouco resistentes, “Pérola” e “Aporé” suscetíveis e “FORT-10” altamente suscetíveis.

Palavras-chave adicionais: Redução do crescimento, resistência, feijoeiro, nematóides das galhas

The bean *(Phaseolus vulgaris L.)*, cultivated in almost all regions of Espirito Santo State, Brazil, is mainly produced by small farmers who have been using traditional cultivars for several generations. These cultivars have genetic variability in several features such as adaptability to ecological conditions, resistance or tolerance to pests, diseases and environmental stresses, plant growth habit, cultural cycle, seed color, brightness and size, and culinary characteristics. Thus, these cultivars are excellent repository of genes of interest for researchers, particularly...
for the use and management of this variability (9).

In the bean crop, there is occurrence of many pathogens that cause significant economic damage. Among the parasites, various species of root-knot-nematodes (Meloidogyne spp.) are quite common, but *Meloidogyne incognita* (Kofoid & White) Chitwood and *M. javanica* (Treub) Chitwood are the most prevalent and damaging species (11) and are thus considered one of the main causes of low yield, especially in areas of predominantly sandy soils and high temperatures (20).

Considered sedentary endoparasites, *Meloidogyne* spp. are among the most successful parasites in the nature (29). These plant-parasitic nematodes penetrate the root system and induce the formation of galls, obstructing the absorption of water and nutrients by the plants (23). Infected plants show reduced growth besides small and yellowish leaves, symptoms that overlap those of mineral deficiency. The grains are small and their production is reduced (12). As noted by Dutra & Campos (8), bean production in the field may be inversely proportional to *M. incognita* population.

Plant resistance has been considered the most desirable tactics in nematode management, especially for the genus *Meloidogyne*, which shows a specialized interaction with their hosts (24). A criterion to evaluate nematode resistance in plants is to measure the nematode reproductive rates in their hosts (26).

In Brazil, there are few cultivars of the genus *Phaseolus* showing moderate resistance to *Meloidogyne* spp.; however, the reproduction of these pathogens is not frequently satisfactorily reduced (18, 19, 20). In United States, Hadisoeganda & Sasser (15) found high resistance in the cultivars Big Boy, Kentucky Wonder and INRA 223 to *M. incognita* and *M. javanica*.

In this context, to achieve a more efficient management of *Meloidogyne* spp., cultivars with effective resistance must be developed. In plant breeding programs, the identification of sources that provide resistant genes is one of the main steps (17). Initially, the breeder usually makes use of existing genes in strains or cultivars, once these sources are more easily accessible, but in some cases genes do not exist or, if present in commercial cultivars, do not give a satisfactory resistance level. In this case, the researcher should use wild germplasm, which has not undergone breeding (17) and is often possession of small farmers linked to the family farm.

Thus way, the aims of the present study were to quantify the damage and to evaluate the resistance levels of common bean genotypes rescued from the South and mountain regions of Espírito Santo State to *M. incognita* race 3 and *M. javanica*.

**Material and Methods**

Bean genotypes cultivated for several years in family farming system were rescued. It is important to note that these genotypes have not been subjected to any official breeding. The collections of these genotypes were kept in the South and mountain regions of Espírito Santo State, Brazil. After the rescue, these genotypes were stored in a cold chamber at the Center of Agrarian Sciences, Federal University of Espírito Santo (CCA-UFES), Alegre, Espírito Santo State, until the beginning of the experiment.

The work was carried out in a greenhouse at CCA-UFES, between January and April 2007. Four genotypes (“FORT-10”, “FORT-13”, “FORT-16” and “FORT-19”) and three cultivars (“Pérola”, “Rico-23” and “Aporé”), also cultivated by farmers from the previously mentioned regions, were tested. The cultivar “Rico-23” was included as a standard susceptible to nematodes (5). Thus, the experiment was carried out in a completely randomized design in a 3 x 7 factorial arrangement (2 nematode species: *M. incognita* race 3 and *M. javanica* + 1 treatment represented by non-inoculated plants x 7 bean genotypes) with 7 replicates. Each plot consisted of one plant per recipient. Non-inoculated plants served as control.

To obtain the inoculum, nematodes were maintained in the roots of tomato (*Solanum lycopersicon* L.) cv. Santa Clara in a greenhouse (21). The substrate was composed of soil collected from an uncultivated area, manually mixed with sand at a ratio of 1:1 (V: V) and sterilized in an autoclave (140°C/1 hour, repeated during three consecutive days). After 60 days, the roots of tomato plants were separated from shoots, washed, cut into 5cm pieces and crushed in a blender using tap water, resulting in the inoculum of both nematode species. From these suspensions, three aliquots of 1 mL each were removed and the eggs + second-stage juveniles (J2) were counted in a Peter’s chamber under an optical microscope.

The bean seeds were pre-germinated in sterile germitest paper rolls placed in a growth chamber at constant temperature of 25°C until radicle emergence (3). Afterwards, these pre-germinated seeds were transferred to plastic pots containing 2000 cm³ of a mixture of soil and sand, at the ratio 2:1 (V: V), previously sterilized in an autoclave, as described before. Two seeds per pot were used and, after the initial growth, one seedling was eliminated one day before the inoculation of plants with nematodes.

Nineteen days after transplanting, each plant received an aqueous suspension containing 2,000 eggs + *M. incognita* race 3 or *M. javanica* J2, applied with a graduated pipette in two 2 to 3 cm deep holes at 3-4 cm from the stem.

Fertilization and pest/disease control of the shoots were made according to the culture needs.

Fifty days after inoculation, the following variables were evaluated: plant height (PHE), number of nodes (NNO), number of trifoliate leaves (NTR), fresh matter weight (FWE) and dry matter weight (DMA) of shoot, root weight (RWE), number of root nodules (NRO) and final nematode population per root system (FPO).

Initially, PHE was determined using a graduated tape. Later, NOS and NFT were counted. To better assess the influence of nematodes on plant growth, the whole shoot was cut and PMF was determined. After weighing, the shoots of each plant were wrapped in paper bags and dried in a force aeration oven at a temperature of 70 °C for 72 hours. Then, the DWE per plant was determined.

To quantify NRD and FPO per plant, the whole root system was carefully washed in water inside a container. After visual counting of NRO, the roots of each plant were cut into pieces of 0.5 cm and crushed in a blender with 200 mL of 0.5% sodium hypochlorite for one minute, passed through a 400 mesh sieve (0.038 mm) and washed in tap water, according to the method of Hussey & Barker (1973), modified by Bonetti & Ferraz (2). From this suspension, three aliquots of 1 mL each were obtained and the eggs + J2 were counted using an optical microscope, resulting in a final nematode population per root system (FPO).

The FPO estimate allowed to determine the reproduction factor (RFA), calculated by the relationship between FPO and the initial population (RF = FPO / IPO), and subsequently the percentage of reproduction (% RRE). Based on % RRE, the bean resistance levels were determined (19) (Table 1).
To meet ANOVA criteria, data were transformed as follows: PHE and FPO (log x), NNO, "WDE, DWE and RWE ("x), NRO (log x + 1), and NRT (data not transformed). To perform the analysis, the software GENES was used, and the averages compared by Tukey test at 5% probability.

RESULTS AND DISCUSSION

The results confirmed that *M. incognita* and *M. javanica* are pathogens that could cause damage to beans (20) (Table 2).

Both nematode species negatively influenced the growth and development of plants compared to the control (non-inoculated plants), except the variables NRO and NNO to accession “FORT 19” (Table 2). The effect of both nematode species on PHE, NNO, NRT and RWE was statistically equal. It was observed an intense reduction in RWE for “FORT 19” when parasitized by *M. javanica*. Freire & Ferraz (11) evaluated the effect of parasitism by *M. incognita*, *M. javanica* and the interaction between these two species on the production of bean cv. “Rico 23”. According to those authors, there were no significant differences between treatments. These results corroborate those obtained in the present study.

When parasitized by *M. incognita*, the cultivars did not differ for any variable when the interaction was significant (Table 2). The same reaction occurred in relation to parasitism by *M. javanica*, except for the variable NRO.

Di Vito et al. (7) also observed the effect of nematode parasitism reducing the growth and development of common beans, corroborating the results of the present study. Those authors, working with a population of *M. javanica* obtained in Italy, reported a reduction in the growth of bean plants cv. Talent (susceptible to nematodes), even when exposed to low levels of nematode population (8 eggs + J2 <sup>3</sup> cm of soil).

Results similar to those found in this study, considering the variable PHE, were obtained by Freire & Ferraz (11), who found significant differences in this characteristic between non-inoculated plants and plants parasitized by *M. incognita* and *M. javanica* and even when both species acted reciprocally.

According to Fortnum et al. (10), PHE reduction in tomato cv. Rutgers due to *M. incognita* parasitism resulted in lower internodes and reduced total fresh biomass as the levels of nematode population increased. Reductions in PHE, NNO, NRT, RWE, FWE and DWE were observed in the bean cultivars in this study (Tables 2 and 3). Ouvir

Damage caused by nematodes could also be observed in the root system of plants. As shown in Table 2, there is a significant reduction in the weight of roots of plants infected by both nematode species. According to Dutra & Campos (8), the largest population of *M. incognita* led to a greater reduction in the root system of bean cv. “Pérola” cultivated under irrigation system.

As described by Davis et al. (6), some organisms can interact with phytonematodes reducing their damage to crops; conversely, some other organisms beneficial to plant growth can be inhibited by the presence of nematodes. According to Hussey & Barker (16), the potential for nodulation in leguminous plants is affected when they
are parasitized by nematodes. In fact, except for accessions “FORT-13” and “FORT-10”, all other cultivars in this study showed reduced NRO when parasitized by *M. javanica*. However, full compromising of nodulation in any cultivar was not observed when plants were inoculated with both nematode species. Freitas et al. (12) reported that nodulation can be stopped or inhibited in the presence of phytonematodes; however, the reduction in nitrogen fixation may not be immediate, but there is an accelerated deterioration of the nodules.

As a consequence of the reduced NRO, the ability of nitrogen fixation by bean plants may be impaired, leading to lower plant development. This fact, associated with the low technological level of farmers in the south of Espírito Santo State, justifies the intended aims and the results obtained in this study.

There was no significant interaction between factors for the variables FWE and DWE. However, for the nematode factor, considering all genotypes and cultivars together, there were significant differences for these variables within the nematode levels (Table 3).

*M. incognita* and *M. javanica* parasitism resulted in less accumulation of fresh and dry matter in the shoots (Table 3). Moura & Moura (18) studied black, white and brindle beans and found no difference between parasitism by *M. incognita* race 1 and by *M. javanica* on the variables shoot weight and root weight compared to control (non-inoculated plants). Unlike the findings of this study, those authors stated that the plants showed a satisfactory growth under all treatments of nematode-cultivar combinations; no difference was observed between the nitrogen content of shoots. It’s important to remind that the experimental conditions of the present work compared to those of Moura & Moura (18) were not the same. Differences between factors such as texture and soil temperature, inoculum level and nematode strain can justify the difference between the results of these studies.

Currently, there is a paucity of researches aiming to quantify the damages caused by nematodes in bean; however, such studies have been conducted with other cultures. Fortnum et al. (10), for example, inoculated tomato plants cv. Rutgers with initial populations of 0 (zero), 1 (one), 10, 50, 100, and 200 (x 1,000) eggs of *M. incognita* per plant. Those authors found that infected plants had lower shoot weight and leaf area.

With respect to control plants, there was a difference between cultivars for the variables PHE, NNO, NRT and RWE, i.e. these variables demonstrate morphological and physiological peculiarities inherent in each cultivar. However, for the variable NRO, all cultivars were similar when not inoculated but had a divergent behavior when parasitized by *M. javanica* (Table 2).

Considering the particularities of each cultivar, the latter were evaluated for resistance level to nematodes (19). Thus, cultivars were divided into three groups in terms of reaction to *M. incognita*, i.e. MS (moderately susceptible), LR (low resistant) and HS (highly susceptible) (Table 4), and also into three groups in terms of reaction to *M. javanica*: LR, SU (susceptible) and HS (Table 5).

Any of the genotypes assessed in the present study behaved as resistant to nematodes. In Brazil there are few works indicating resistance sources to *Meloidogyne* spp., within the genus *Phaseolus*, but there are references for only a few cultivars with moderate resistance, without, however, satisfactorily reducing the parasite reproduction (5, 18, 19, 20).

As shown in Tables 4 and 5, except cv. “Rico 23”, other cultivars behaved differently for the two nematode species. One example is accession “FORT 10” classified as MR when parasitized by *M. incognita* but behaving as HS to *M. javanica*. Corroborating these results, Pedrosa et al. (20) studied 162 varieties and reported a different behavior for resistance to *M. incognita* race 1 and *M. javanica* in some of the evaluated materials. In the study of Moura & Moura (18), black and white genotypes, when inoculated with *M. incognita* race 1, behaved as HS, and these same genotypes were classified as HS and S, respectively, when inoculated with *M. javanica*.

According to Carneiro & Ferraz (4), varieties identified as resistant *M. incognita* populations from a given region may have different reactions when exposed to parasitism of populations from other areas. Vieira (28) found that 18 cultivars, including “Manteigão fosco” and “Rico 23”, showed varying levels of susceptibility to *M. incognita*. Such references justify the behavior of cv. “Rico 23”, considered susceptible to *Meloidogyne* sp., as LR to both *Meloidogyne* species.

### Table 3 - Fresh weight (RWE) and dry weight (DWE) of shoots of four genotypes and three commercial cultivars of common bean (*Phaseolus vulgaris* L.) infected or not by *Meloidogyne incognita* race 3 or *M. javanica*.

<table>
<thead>
<tr>
<th>Variables</th>
<th><em>M. incognita</em> race 3</th>
<th>Non-inoculated</th>
<th><em>M. javanica</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>RWE</td>
<td>0.91 b</td>
<td>1.57 a</td>
<td>0.86 b</td>
</tr>
<tr>
<td>DWE</td>
<td>0.45 b</td>
<td>1.18 a</td>
<td>0.43 b</td>
</tr>
</tbody>
</table>

¹ Means followed by same letter in the row are not statistically different according to Tukey’s test at 5% probability.

### Table 4 - Reproduction factor (RF), percentage of reproduction (%R) and reaction of four genotypes and three cultivars of common bean (*Phaseolus vulgaris* L.) to parasitism by *Meloidogyne incognita* race 3, according to Moura & Regis (1987).

<table>
<thead>
<tr>
<th>Cultivar (c)/genotype (g)</th>
<th>RF</th>
<th>% R*</th>
<th>Reaction*</th>
</tr>
</thead>
<tbody>
<tr>
<td>FORT-10 (g)</td>
<td>1.1</td>
<td>90.35</td>
<td>MR</td>
</tr>
<tr>
<td>FORT-13 (g)</td>
<td>1.5</td>
<td>86.84</td>
<td>MR</td>
</tr>
<tr>
<td>Aporé (c)</td>
<td>2.0</td>
<td>82.45</td>
<td>MR</td>
</tr>
<tr>
<td>FORT-16 (g)</td>
<td>2.1</td>
<td>81.57</td>
<td>MR</td>
</tr>
<tr>
<td>Rico 23 (c)</td>
<td>4.1</td>
<td>64.03</td>
<td>LR</td>
</tr>
<tr>
<td>Pêrola (c)</td>
<td>5.3</td>
<td>53.50</td>
<td>LR</td>
</tr>
<tr>
<td>FORT-19 (g)</td>
<td>11.4</td>
<td>0.00</td>
<td>HS</td>
</tr>
</tbody>
</table>

* MR = moderately resistant; LR = low resistant; HS = highly susceptible.

### Table 5 - Reproduction factor (RF), percentage of reproduction (%R) and reaction of four genotypes and three cultivars of common bean (*Phaseolus vulgaris* L.) to parasitism by *Meloidogyne javanica*, according Moura & Regis (1987).

<table>
<thead>
<tr>
<th>Cultivar (c)/genotype (g)</th>
<th>RF</th>
<th>% RFR*</th>
<th>Reaction*</th>
</tr>
</thead>
<tbody>
<tr>
<td>FORT-19 (g)</td>
<td>0.9</td>
<td>74.28</td>
<td>LR</td>
</tr>
<tr>
<td>Rico 23 (c)</td>
<td>1.4</td>
<td>60.00</td>
<td>LR</td>
</tr>
<tr>
<td>FORT-16 (g)</td>
<td>1.4</td>
<td>60.00</td>
<td>LR</td>
</tr>
<tr>
<td>FORT-13 (g)</td>
<td>1.6</td>
<td>54.28</td>
<td>LR</td>
</tr>
<tr>
<td>Pêrola (c)</td>
<td>1.8</td>
<td>48.57</td>
<td>SU</td>
</tr>
<tr>
<td>Aporé (c)</td>
<td>2.5</td>
<td>28.57</td>
<td>SU</td>
</tr>
<tr>
<td>FORT-10 (g)</td>
<td>3.5</td>
<td>0.00</td>
<td>HS</td>
</tr>
</tbody>
</table>

*LR = low resistant; SU = susceptible; HS = highly susceptible.
used in this study. This difference in the behavior of host plants and the different strains of nematodes can be explained by the genetic variability showed by these pathogens, once the spectrum of virulence or avirulence within and between species and populations of *Meloidogyne* spp. is complex (25). Those authors state that the virulence or avirulence of nematodes affect the frequency of infection of a host for a *Meloidogyne* isolate and can be measured by the proportion of second-stage juveniles (J2) that develop in pregnant females and the number of eggs per egg mass of nematodes. Thus, according to those authors, virulence or avirulence affects plant fertility and primary infection by the nematode.

Similarly to the common bean, there are few studies with cowpea bean indicating efficient sources of resistance to root-knot nematodes. In studies conducted by Wanderley et al. (30), all evaluated cowpea beans were susceptible to *M. javanica*, such as cultivars “Conjunha” and “Canapuí”, which exhibited the highest RF values, being considered the most susceptible. Similarly to Wanderley et al. (30), Goulart et al. (13) also concluded that all cowpea beans tested in their work behaved as susceptible to *M. javanica* and *M. incognita*.

According to Simão et al. (27), cv. “Pérola” behaved as HS even at lower *M. javanica* inoculum densities. Perre & Santos (22) obtained similar results when the susceptibility of bean genotypes inoculated with this nematode was high. The same was not found in this work, in which cv. “Pérola” behaved as S to *M. javanica* and as LR to *M. incognita*. However, such data are consistent with the results obtained by those authors since they concluded that cvs. “Pérola” and “lapar 81” may not have significant yield losses, while allowing the reproduction of the nematode, which explains the low susceptibility observed in this study compared to the others. It is noteworthy that fact that such differences may have been caused by numerous factors that influence the ability of resistance expression in genotypes, for example, the different temperature conditions under which the experiments were carried out. According to Alves & Campos (1), temperature is a critical factor influencing the expression of plant resistance to nematodes.

In areas infested by nematodes, special care must be taken in the choice of cultivars. The use of cultivars resistant to nematodes in crop rotation systems prevents future damage in the most susceptible botanical species. Simão et al. (27) assume that the use of cv. “Pérola” and “lapar 81” in soil with the presence of *M. javanica* and in regions of predominantly high temperatures can lead to increased population of nematodes in the cultivation area. Dutra & Campos (8) confirmed these results also working with cv. “Pérola” in soils infested with *M. incognita*. Therefore, based on the results of this study and others available in the literature, planting cv “Pérola” should be avoided since this genotype allows high multiplication of *M. incognita* race 3 and *M. javanica*.

Overall, the bean genotypes used by farmers in the southern region of Espírito Santo State have moderate and low levels of resistance to nematodes. Results obtained by Di Vito et al. (7) and Greco et al. (14) are in agreement with these findings. According to those authors, several bean varieties used by Italian farmers are not resistant to nematodes and are severely damaged by *M. javanica* species commonly found in cultivated fields.

Although yield was not evaluated in the present study, four genotypes were classified as MR (“FORT-10”, “FORT-13”, “FORT-16”) and “Aporé” indicating possible sources of resistance to *M. incognita* race 3. However, in order to have full consolidation of results, the experiment should be repeated in the field.

The genotypes included in this study are already adapted to the soil and climate of the south of Espírito Santo State and have been cultivated for years by familiar farmers. Considering that some of these genotypes showed resistance to *Meloidogyne* spp., the information obtained in this work can be useful to decide which bean genotypes should be used in breeding programs in order to find resistance to nematodes.

REFERENCES


