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Soybean development under different *Aphelenchoides besseyi* and *Meloidogyne incognita* populations

Desenvolvimento da soja sob diferentes populações de *Aphelenchoides besseyi* e *Meloidogyne incognita*

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Highlights

The presence of phytonematodes reduces phytotechnical components of soybean.
High populations of *M. incognita* inhibited the presence of *A. besseyi.*
A. besseyi population negatively correlated with the number of pods and grains.

Abstract

The green stem *Aphelenchoides besseyi* and gall *Meloidogyne incognita* phytonematodes cause soybean yield losses. Thus, the objective of this study was to evaluate the effects of different populations of *A. besseyi* and *M. incognita* on soybeans. The experiment had 10 treatment groups [T_1: control, T_2: *A. besseyi* (500), T_3: *M. incognita* (2,000), T_4: *A. besseyi* (250) + *M. incognita* (2,000), T_5: *A. besseyi* (500) + *M. incognita* (2,000), T_6: *A. besseyi* (250) + *M. incognita* (4000), T_7: *A. besseyi* (500) + *M. incognita* (4,000) individuals, T_8: *A. besseyi* (250) + *M. incognita* (8,000), T_9: *A. besseyi* (500) + *M. incognita* (5,000), and T_{10}: *A. besseyi* (1,000) + *M. incognita* (8,000) individuals] with six replicates. Inoculations were done on day 10 after soybean germination, and evaluations were conducted after 60 d. Plant height, shoot fresh weight, root fresh weight, number of pods (NP), pod fresh weight, number of grains (NG), grain yield (GY), amount of *A. besseyi* in the shoot, and *M. incognita* in the roots were analyzed. The 500 *A. besseyi* + 8,000 *M. incognita* treatment reduced all variables and led to considerable damage to soybean development. In addition, GY, NG, and NP were reduced in the different nematode population density groups, influencing soybean agronomic characteristics.

Key words: Simultaneous infection. Green stem nematode. Root-Knot nematode. Yield componentes.

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Resumo

Entre as espécies de fitonematoides que provocam perdas de produção à cultura da soja estão os nematoides da haste verde (Aphelenchoides besseyi) e o das galhas (Meloidogyne incognita). Diante disso, o objetivo deste trabalho foi estudar os efeitos de diferentes níveis populacionais de A. besseyi e M. incognita na soja. O experimento foi realizado com nove combinações de diferentes níveis populacionais dos nematoides (T1 – Controle não inoculado, T2 - A. besseyi 500 indivíduos, T3 - M. incognita 2000 indivíduos, T4 - A. besseyi + M. incognita 250 + 2000 indivíduos, T5 - A. besseyi + M. incognita 500 + 2000 indivíduos, T6 - A. besseyi + M. incognita 250 + 4000 indivíduos, T7 - A. besseyi + M. incognita 500 + 4000 indivíduos, T8 - A. besseyi + M. incognita 250 + 8000 indivíduos, T9 - A. besseyi + M. incognita 500 + 8000 indivíduos e T10 - A. besseyi + M. incognita 1000 + 8000 indivíduos) e seis repetições. As variáveis analisadas foram: altura de planta, massa fresca da parte aérea (MPA), massa fresca de raízes (MR), número de vagens (V), massa fresca de vagens (MV), número de grãos (G), massa fresca de grãos (MG), quantidade de A. besseyi na parte aérea e de M. incognita nas raízes. O mapa de calor indicou que populações iniciais inoculadas de 500 A. besseyi + 8000 M. incognita apresentou maiores danos ao desenvolvimento da soja, reduzindo todas as variáveis analisadas. As variáveis MG, G e V reduziram na presença de diferentes densidades populacionais das espécies de fitonematoides nematoides, influenciando negativamente o desenvolvimento da soja.

Palavras-chave: Infecção conjunta. Nematoide da haste verde. Nematoide de galhas. Componentes fitotécnicos.

Introduction

Soybean (*Glycine max* (L.) Merrill) is a multipurpose crop of great economic importance worldwide. Its products and byproducts are widely used in the chemical and food industries and the production of biofuels. Brazil is the world's leading soybean producer and major exporter (Companhia Nacional de Abastecimento [CONAB], 2020). However, an increased cultivation of soybean combined with poor management practices has increased the spread of pests and diseases, including phytonematodes, leading to huge economic losses (Dias et al., 2010).

More than 100 species of nematodes that cause grain losses in soybean have been reported (Dias et al., 2010). However, losses may vary depending on the pathogen population density, resistance of the cultivar, and soil and climatic conditions. *Aphelenchoides besseyi* and *Meloidogyne incognita* are among the phytonematode species that cause the greatest losses in soybean production.

A. besseyi has been a nuisance to the Brazilian agricultural sector, and the primary symptoms shown by plants infested by it are leaf retention, thickened nodes, and low senescence (Meyer et al., 2017). *A. besseyi* is a non-obligatory phytoparasite: it survives as a mycofire, feeding on fungi that decompose soil organic matter in the absence of host plants (Favoreto et al., 2011; Jesus & Cares, 2016).

The symptoms caused by nematodes of the *Meloidogyne* genus are stunting of plants with dwarfism, chlorosis, premature ripening, and intense abortion of pods (Dias et al., 2010). *Meloidogyne* nematodes are sedentary endoparasites that permanently feed on host plants, forming galls and making it difficult for plants to absorb water and nutrients (A. M. R. Almeida et al., 2005; Godoy et al., 2016).

The objective of this study was to investigate the individual and combined effects of different population levels of *A. besseyi* and *M. incognita* on soybean development and reproduction.

Materials and Methods

This study was carried out under greenhouse conditions at Embrapa Soja, Londrina, Paraná State, Brazil. The experiment was conducted with constant nebulization periods of 15 s every 30 min and at an average temperature of 26 °C (± 2°C). Sixty pots (3.5 L) were filled with a substrate previously autoclaved at 120 ºC for 1 h composed of sand and clay at a ratio of 3:1. Two BRS 284RR soybean seeds susceptible to the two phytonematode species were sown in each pot. Thinning was done 10 days after seedling emergence, leaving 1 plant per pot. A completely randomized design with 10 treatments [T_1: control; T_2: *A. besseyi* (500); T_3: *M. incognita* (2,000); T_4: *A. besseyi* (250) + *M. incognita* (2,000); T_5: *A. besseyi* (500) + *M. incognita* (2,000); T_6: *A. besseyi* (250)+ *M. incognita* (4,000); T_7: *A. besseyi* (500) + *M. incognita* (4,000); T_8: *A. besseyi* + *M. incognita* (250) + (8,000); T_9: *A. besseyi* (500) + *M. incognita* (8,000); and T_{10}: *A. besseyi* (1,000) + *M. incognita* (8,000 individuals)], with 6 replicates was used in this experiment.

A. besseyi specimens were extracted from infested soybean plants using the method described by Coolen and D'Herde (1972) and multiplied in vitro. First, the multiplication of this species was done by selecting 20 individuals (15 females and 5 males) in 0.1% ampicillin solution under a stereoscopic microscope. Next, the nematodes were transferred into Petri dishes with *Fusarium sp.* colonies cultured on potato-dextrose-agar media for approximately 5 d (Favoreto et al., 2011). Finally, the *A. besseyi* were placed in biochemical oxygen demand incubation chambers at 25 ±1 °C for approximately 30 d in the dark (Favoreto et al., 2011) until inoculation of plants in the greenhouse. The inoculum was prepared by washing the droplets on the lid of the Petri dishes with distilled water, as described by Meyer et al. (2017).

The *M. incognita* inoculum was obtained from infested soybean roots and processed as described by Bonetti and Ferraz (1981). Briefly, roots were cut into pieces of approximately 1 cm in length, ground in a blender using a 0.5% solution of sodium hypochlorite for 20 s, and the material was sieved by pouring it onto a 200-mesh sieve over a 500-mesh sieve. The material retained was collected and observed under an optical microscope at 40× magnification to count the number of nematodes using a Peters counting chamber and a unit cell counter.

The suspension concentration of nematode species was calibrated considering the initial population of each treatment and was adjusted to provide the same volume to all plants. Inoculation was done 10 d after soybean emergence by depositing an aliquot of the inoculum in an open hole in the soil next to the plant's stem using a 1,000-µL micropipette.

Sixty days after inoculation, the fresh weights of the shoot (SFW), root (RFW), and pod (PFW); number of pods (NP); plant height (PH); number of grains (NG); grain yield (GY); total number of *A. besseyi* in shoots (NAS); and *M. incognita* in roots (NMI) were measured.

After measuring the plant height, shoots were separated from the roots, placed in plastic bags, and taken to the laboratory. The roots and shoots were washed with running water, dried on paper towels, and weighed to determine the RFW and SFW. Subsequently, roots and shoots were processed according to the procedures described by Bonetti and Ferraz (1981) and Coolen and D'Herde (1972), respectively. Finally, the nematodes in the samples were observed under an optical microscope at 10× magnification using a Peters camera.

To evaluate the effect of the combined inoculation of *A. besseyi* and *M. incognita* populations on plants, the reproduction rates of parasites in plants were determined by calculating the "Reproduction Factor" (RF) using the formula: RF = Number of eggs and J_2 obtained by root system / Number of eggs and J_2 used in inoculation (Cook & Evans, 1987), where J_2 is the second stage of the infective juvenile stage.

Data were subjected to normality (Shapiro–Wilk) and homogeneity (Bartlett) tests. Furthermore, analysis of variance and the F-test were conducted, and a heat map was generated using the Pearson's correlation coefficient.

Results and Discussion

The root-knot nematode, *M. incognita,* exhibited the highest reproduction rate (Figure 1), except when inoculated in combination with the lowest and median levels of *A. besseyi* (T_8 and T_9). After inoculating the highest concentrations of both species, the RF for *A. besseyi* was approximately zero (T_{10}), indicating a competition between the two species.

The biological differences between the species may explain the lower RF averages in A. besseyi populations than those in M. incognita. M. incognita is an aggressive species; it penetrates the roots in the infective, J2 stage (Dias et al., 2010). In addition, females remain inside the roots until the end of the life cycle, inducing hypertrophy and hyperplasia in tissues around the feeding site (coenocytes), which results in the formation of root galls (Moura, 1996) and death of the plants in extreme cases (Dias et al., 2010). The life cycle of the phytonematodes is 4 weeks and can be extended under unfavorable temperature conditions (Costa et al., 2000). The hatching of M. incognita eggs is influenced by humidity and temperature (Dias et al., 2010). However, the current experiment was carried out under optimum temperature and humidity for M. incognita for the entire cycle of its development (Almeida & Seixas, 2010). Therefore, M. incognita had greater egg hatching and was more aggressive than A. besseyi; hence possibly it penetrated the roots, moved internally in tissues, and passed through the stem to the inflorescence of the plant where it fed and completed its life cycle (Favoreto & Meyer, 2019). M. incognita can multiply in many species of fungi, with rates ranging from 1 to 102 times over 15 days (Marques & Huang, 1984).

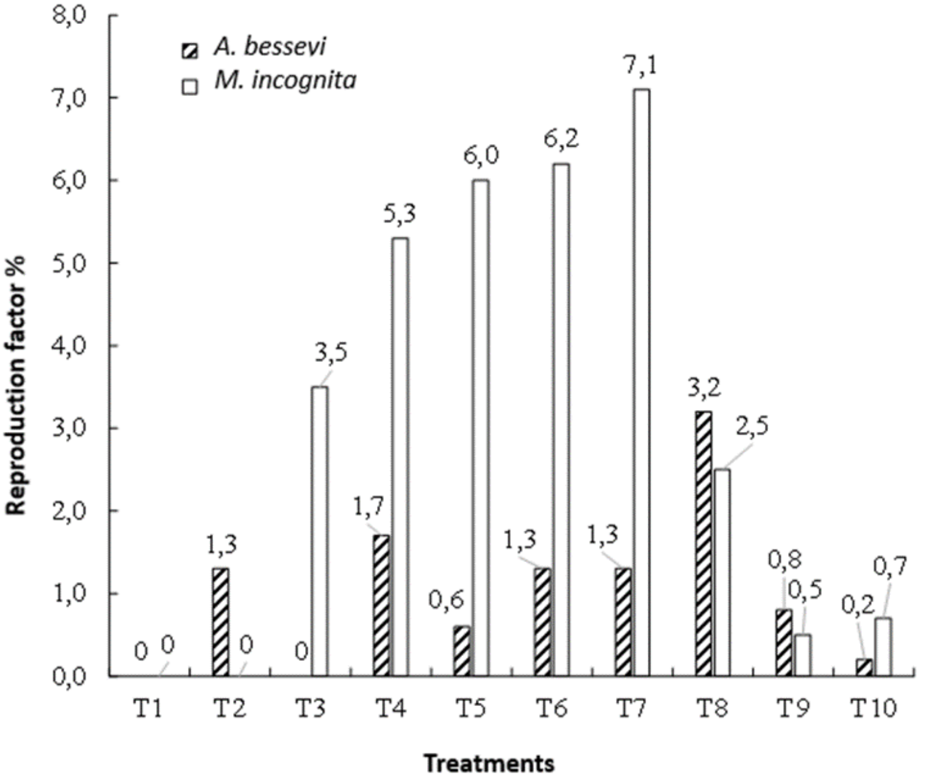

Figure 1. Reproduction factor (RF) at 60 d after inoculation in 10 treatments. T_1: Control; T_2: *A. besseyi* (500); T_3: *M. incognita* (2,000); T_4: *A. besseyi* (250) + *M. incognita* (2,000); T_5: *A. besseyi* (500) + *M. incognita* (2,000); T_6: *A. besseyi* (250) + *M. incognita* (4.000); T_7: *A. besseyi* (500) + *M. incognita* (4,000); T_8: *A. besseyi* (250) + *M. incognita* (8,000); T_9: *A. besseyi* (500) + *M. incognita* (8,000); and T_{10}: *A. besseyi* (1,000) + *M. incognita* (8,000). Figures in parenthesis indicate the number of individuals.

The initial nematode population used in the T9 treatment caused a decrease in RFW and SFW; consequently, these plants showed lower NP, NG, PFW, and GY than the plants with other treatments (Table 1). After 60 d of nematode inoculation, PH ranged from 24.8 to 44.7 cm, with an average of 39.1 cm (Table 1), consistent with the results of Almeida et al. (2016). In the current study, plants with better SFW development produced more pods and grains (Table 1).

Asmus and Ferraz (2001) revealed that the effects of parasitism on RFW increased with reduced nematode population and vice versa in roots infected with root-knot nematodes. Dias et al. (2010) and Rosa (2010) reported that plants infested with nematodes had poor RFW and more superficial roots. A low RFW may result from severe M. incognita parasitism, compromising root cellular tissues and lowering RFW values. The presence of A. besseyi alone did not decrease NP, FPW, NG, and GY, like the control treatment at 60 d after planting (Table 1), contrary to the results obtained by Favoreto and Meyer (2019).

Table 1
Clay, pH and chemical composition of the soil used for wheat cultivation

Treatments	HP (cm)	SFW (g)	RFW (g)	NAS (n)	NMR (n)	NP (n)	PFW (g)	NG (n)	GY (g)
T1	41.4a	30.7a	28.0a	0.0c	0.0d	38.0a	32.5a	85.5a	15.8a
T2	43.3a	34.2a	36.3a	20.7a	0.0d	34.5a	38.3a	94.5a	19.6a
T3	37.9a	22.8b	26.2b	0.0c	297.6c	23.5b	22.8b	62.0b	12.8b
T4	38.0a	21.9b	24.7b	18.6a	450.4b	20.8b	21.3b	56.0b	11.4b
T5	42.1a	23.2b	31.8a	13.6b	401.0b	22.5b	21.0b	53.8b	10.5b
T6	40.0a	34.0a	28.7a	15.1b	113.4c	24.5b	25.5b	68.0b	12.2b
T7	38.6a	27.9a	31.7a	20.9a	879.8a	22.3b	33.6a	55.8b	11.6b
T8	40.2a	26.0a	30.0a	27.9a	705.7a	21.3b	18.8b	43.3b	8.9b
T9	24.8b	19.8b	22.5b	25.2a	187.5c	9.2c	8.1c	18.5c	3.7c
T10	44.7a	23.0b	29.8a	8.1b	196.2c	28.5b	23.2b	59.2b	12.3b
Mean	39.1	26.4	29.0	15.0	323.2	24.5	24.5	59.7	11.9

PH: plant weight; SFW: shoot fresh weight; RFW: root fresh weight; NAS: total number of *Aphelenchoides besseyi* in shoots; NMI: total number of *Meloidogyne incognita* in roots; NP: number of pods; PFW: pod fresh weight; NG: number of grains; GY: grain yield; T_1: control; T_2: *A. besseyi* (500); T_3: *M. incognita* (2,000); T_4: *A. besseyi* (250) + *M. incognita* (2,000); T_5: *A. besseyi* (500)+ *M. incognita* (2,000); T_6: *A. besseyi* (250) + *M. incognita* (4,000); T_7: *A. besseyi* (500) + *M. incognita* (4,000); T_8: *A. besseyi* (250) + *M. incognita* (8.000); T_9: *A. besseyi* (500) + *M. incognita* (8,000); and T_{10}: – *A. besseyi* (1,000) + *M. incognita* (8,000 individuals). Means followed by different letters in the same column differ by 5% using the Scott–Knott test.

The correlations among NAS and PH, SFW, RFW, NP, PFW, NG, and GY were inversely proportional (Figure 2), similar to the correlations between NMI in roots and the same variables. Therefore, the presence of NAS and NMI decreased the other variables studied, as shown in Table 1.

The negative correlations between the presence of A. besseyi and the PH × SFW combination (Figure 2) may be the result of stunting caused by nematodes (Favoreto & Meyer, 2019), as reported by Neves et al. (2011) in strawberry plants (Fragaria × ananassa). In addition, the Pearson's correlation coefficient (Figure 2) revealed that PH was more influenced by the presence of *A. besseyi* (-0.32) than M. incognita (-0.07). In addition, high concentrations of NMI used in the T10 treatment did not reduce PH (Table 1).

Furthermore, the presence of A. besseyi in the aerial parts of plants had a greater negative correlation with NP, PFW, NG, and GY (-0.28, -0.18, -0.27, and -0.25, respectively) than that in M. incognita roots (-0.11, -0.07, -0.12, and -0.1, respectively). According to Vilas-Boas et al. (2002) and Dias et al. (2010), root-knot nematodes reduce plant development, SFW, and RFW under severe infestation, consistent with the results of Favoreto and Meyer (2019) for A. besseyi.

However, positive correlations can be classified according to their magnitude (Carvalho et al., 2004). In the current study, positive correlations ranged from medium to strong for HP × RFW (0.55), HP × SFW (0.49), and RFW × SFW (0.69) (Figure 2). This is because good root development promotes the formation of the aerial parts of the plant (Almeida et al., 2016). Accordingly, the positive correlations NP × PFW (0.71) and NG × GY (0.91) were classified as strong and extremely strong relationships, respectively (Figure 2), demonstrating a high degree of dependence between both variables, consistent with the results of Carvalho et al. (2004).

Figure 2. Pearson's correlation coefficient among the variables analyzed. PH: plant height; SFW: shoot fresh weight; RFW: root fresh weight; NAS: total number of A. besseyi in shoots; NMI: total number of Meloidogyne incognita in roots; NP: number of pods; PFW: pod fresh weight; NG: number of grains; GY: grain yield.

Analysis of the averages of the variables in the heat map (Figure 3), based on visual symptoms described by Favoreto and Meyer (2019) in plants infected with A. besseyi, showed that T9 led to the greatest damage to soybean development compared with other treatments, as it showed reduction in all variables (Figure 4).

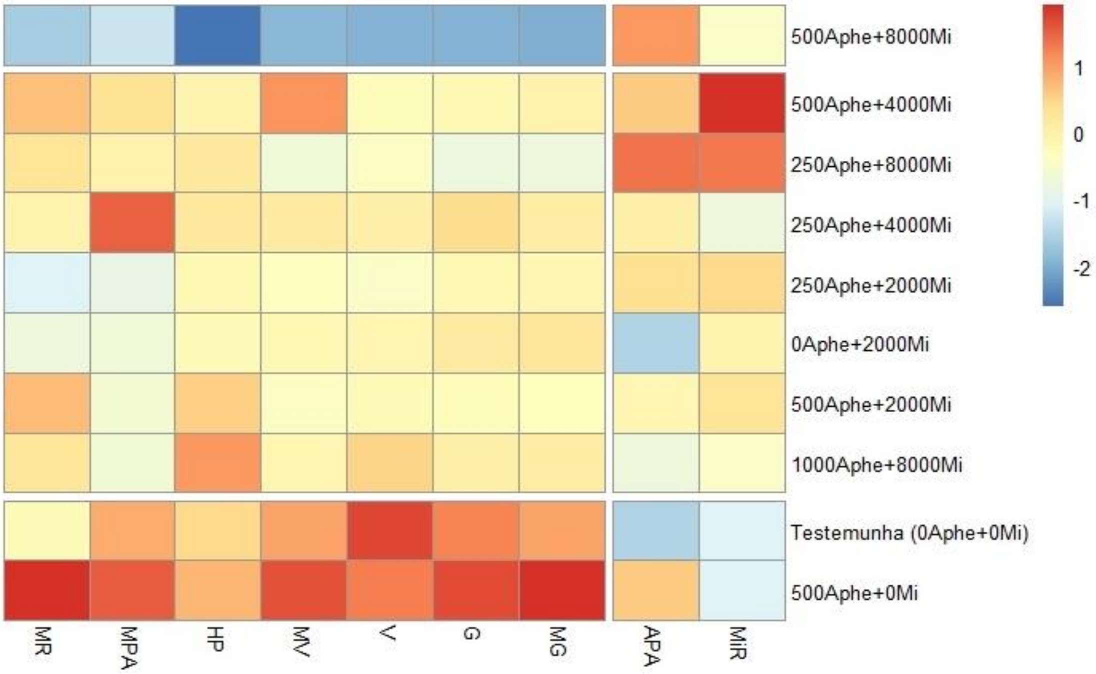

Figure 3. Heat map of the averages of the variables analyzed between treatments. PH: plant height; SFW: shoot fresh weight; RFW: root fresh weight; NAS: total number of A. besseyi in shoots; NMI: total number of Meloidogyne incognita in roots; NP: number of pods; PFW: pod fresh weight; NG: number of grains; GY: grain yield.

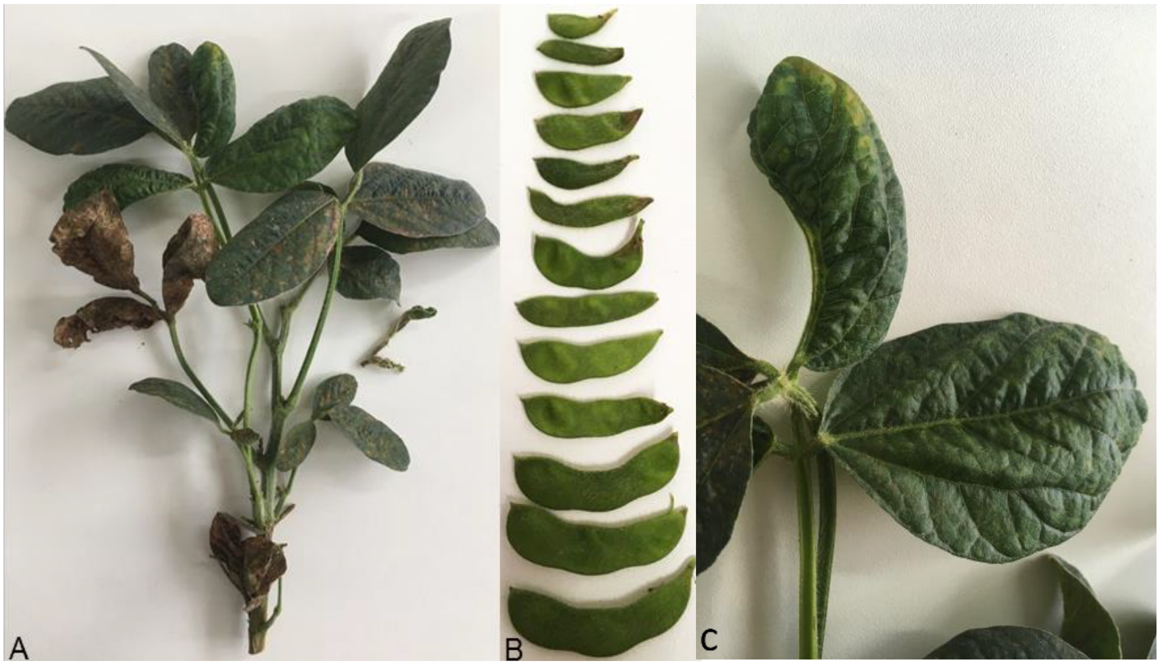

Figure 4. Soybean shoot symptoms (A), pods (B), leaf wilting (C) under high populations of Aphelenchoides besseyi and Meloidogyne incognita.

However, T_2 had values close to those of T_1 (Figure 3). Even with the smallest size of plants, the presence of *A. besseyi* can lead to an increase in SFW by thickening stems, nodes, and internodes (Favoreto & Meyer, 2019). In addition, infection leads to the abortion of flowers and pods, and bean pods remain green, immature, and rot over time (Meyer et al., 2017).

In T_{10}, the variables under study had no significant differences, even with the highest levels of inoculum of both species (Figure 3), consistent with the results in Figure 1. This may probably be due to competition between and within the two nematode species. According to the correlation coefficients (Figure 2), the two nematode species were positively correlated with each other but negatively correlated with the phytotechnical components.

Meyer et al. (2017) reported that *A. besseyi* causes a high reduction in productivity due to high abortion rates of flowers and pods. In addition, Carneiro et al. (2019), in an experiment with *M. incognita,* recorded a GY reduction of up to 52%. Therefore, it can be inferred that simultaneous infestation with the two species impairs the development of plants and negatively affects soybean GY.

Conclusions

The two phytonematodes, regardless of the degree of infection, negatively influence the phytotechnical components of soybean. Under the conditions studied, *M. incognita* was more harmful to soybean development than *A. besseyi.*

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