v. 3, n. 11, 2023

Journal of Agricultural Sciences Research

BIOTIC STRESS CAUSED BY *MELOIDOGYNE ENTEROLOBII* IN PHYSIOLOGY OF GUAVA TREE TREATED WITH *BACILLUS METHYLOTROPHICUS*

Karoliny de Almeida Souza
Universidade de Brasília, Phytopathology Department
Brasília-DF
0000-0001-6736-160X

Nadson de Carvalho Pontes
Instituto Federal Goiano Campus Morrinhos
Morrinhos-GO
0000-0003-2850-8415

Cleber Furlanetto
Universidade de Brasília, Phytopathology Department
Brasília-DF
0000-0003-0575-0487

Juvenil Enrique Cares
Universidade de Brasília, Phytopathology Department
Brasília-DF
0000-0003-3325-5221

Abstract: The interference of nematodes in the physiology of plants is little studied, as well as the ability of rhizobacteria to mitigate the physiological damage caused by nematodes. The objectives of this research were to monitor physiological changes in guava plants parasitized by *Meloidogyne enterolobii* and treated with bionematicide, active ingredient: *Bacillus methylotrophicus* (B.met) and to evaluate the efficiency of the bionematicide in controlling the nematode. The experiment was installed in a greenhouse and the treatments represented by: 1) 'Pedro Sato´ guava not inoculated and not treated with B. met (control); 2) 'Paluma' guava not inoculated and not treated with B. met (control); 3) `Pedro Sato´ not inoculated and treated with B. met; 4) `Paluma´ not inoculated and treated with B. met; 5) `Paluma´ inoculated and not treated with B. met; 6) `Pedro Sato´ inoculated and not treated with B. met; 7) `Pedro Sato´ inoculated and treated with B. met and 8) `Paluma´ inoculated and treated with B. met. Four applications of bionematicide were performed at a dosage of 3 mL/L of water per plant. Between the second and third application, the plants were inoculated with 5000 eggs and juveniles of M. enterolobii. Seven assessments of physiological parameters were performed. At 132 days after inoculation (DAI) the roots were weighed and the gall index, egg mass index and reproduction factor were determined. *Meloidogyne enterolobii* and B. met combined reduced the stomatal conductance and transpiration at 26 and 44 DAI, respectively. Photosynthesis was lower at 26 DAI in treatments that received the nematode and bacteria combined. *Meloidogyne enterolobii* and B. met isolated and combined reduced water use efficiency at 26 and 44 DAI. In root mass there was no difference between treatments. Gall index, egg mass index and reproduction factor were higher in treatments that received nematodes, regardless of the application with the bacteria.
Keywords: alternative control, biological control, photosynthesis, root-knot nematode, rhizobacteria

INTRODUCTION

The guava tree (*Psidium guajava L.*) belongs to the *Myrtaceae* family and is native to tropical America and, among the 150 species of the genus, it is considered the species of greatest economic importance. Guava is among the most nutritious fruits, being rich in vitamin C and a source of vitamins A, B1, B2 and B3 (Pereira & Nachtigal, 2002; Oliveira et al., 2012).

A species of root-knot nematode, identified as *Meloidogyne mayaguensis*, which later became synonymous with *M. enterolobii*, is currently the main phytosanitary problem for guava in Brazil (EPPO, 2023). Meloidogyne enterolobii causes significant losses in production and many times, the producer is obliged to eradicate the orchard, because once present in the area, the nematode control is not efficient (Pereira et al., 2009). *Meloidogyne enterolobii*, in addition to being a polyphagous nematode, it is an aggressive species and differs from other species by being able to overcome the resistance of dominant genes used to control *Meloidogyne spp.*, such as the Mh gene in potatoes, the Mir1 gene in soybeans, the N gene in pepper, the Mi gene in tomato (Abrão & Mazzafera, 2001; Kiewnick et al., 2009).

The main direct symptom in plants infected by *Meloidogyne spp.* is the formation of galls on the roots (Collett et al., 2021). The galls influence the uptake and flow of water from the roots to the shoot, reduce water conductivity, induce morphological changes in the xylem and phloem cells, and make the functioning of conductive vessels unfeasible and, as a consequence, the plant suffers water stress. In addition, the presence of the nematode can trigger physiological

and biochemical changes in plants. All these modifications are able to induce changes in the optimal conditions of photosynthetic rate, stomatal conductivity and transpiration rate (Abrão & Mazzafera, 2001; Domiciano et al., 2009; Azam et al., 2011; Strajnar et al., 2012).

In order to seek new means of controlling nematodes, products based on fungi and bacteria with nematicidal properties and which, in addition, induce resistance mechanisms in the plant, have been growing in importance and use in the world (Mioranza et al., 2018). *Bacillus spp.* are part of the complex of rhizobacteria species that act mainly in the region with the highest concentration of roots and have negative effects against phytopathogenic organisms. Rhizobacteria can mitigate changes in photosynthesis, stomatal conductance and transpiration induced by root-knot nematodes, because they can contribute to an increase in the amount of photosynthetic pigments by protecting the biochemical mechanisms involved in the synthesis of the photosynthetic process and improving the absorption and translocation of nutrients by the plant (Abd-El-Khair et al., 2019; Khanna et al., 2019).

Knowing the ability of *M. enterolobii* to influence the photosynthetic rate, stomatal conductance and transpiration is important since a large part of the dry matter content and productivity are determined by the photosynthetic machinery (Dalastra et al., 2014). There are still no studies that seek to understand the capacity of the nematode to interfere in these physiological processes, as well as the treatment with *Bacillus* can counteract the negative effects of the nematode on guava. The objectives of this research were to monitor physiological changes in guava plants parasitized by the nematode *Meloidogyne enterolobii* and treated with the bionematicide Onix©, active ingredient *Bacillus methylotrophicus* (B.met) by monitoring stomatal conductance, efficiency in water use, photosynthesis and transpiration and evaluating the efficiency of the bionematicide in controlling the nematode.

MATERIAL AND METHODS

MULTIPLICATION OF *MELOIDOGYNE ENTEROLOBII*

A population of *M. enterolobii* from the Department of Phytopathology of ``Universidade de Brasília`` maintained in tomato (*Solanum lycopersicum*) cv. Santa Clara was used in this study. Before inoculation, this population was multiplied in tomato for three months under greenhouse conditions. The nematode was identified by the esterase phenotype technique (Carneiro & Almeida, 2001) and through specific primers MK7-F and MK7-R (Tigano et al., 2010).

TREATMENTS AND EXPERIMENTAL DESIGN

The guava cultivars Paluma and Pedro Sato were evaluated, distributed in 8 treatments represented by: 1) `Pedro Sato´ not inoculated and not treated with B. met (control); 2) `Paluma´ not inoculated and not treated with B. met (control); 3) `Pedro Sato´ not inoculated and treated with B. met; 4) `Paluma´ not inoculated and treated with B. met; 5) `Paluma´ inoculated and not treated with B. met; 6) `Pedro Sato´ inoculated and not treated with B. met; 7) `Pedro Sato´ inoculated and treated with B. met and 8) `Paluma´ inoculated and treated with *B.met*. Clonal seedlings were obtained from a nursery located in the municipality of Morrinhos, GO registered with the Ministry of Agriculture, Livestock and Supply.

The experiment was installed in a greenhouse and conducted from January 2021 to July 2021. A completely randomized design

was used, with five replications, with the experimental unit represented by one plant per pot. The seedlings were transplanted into polyethylene bags measuring 35 x 40cm, with an approximate volume of 11L, filled with autoclaved soil in a 2:1 ratio (sand: soil).

BACTERIAL APPLICATIONS, NEMATODE INOCULATION AND EVALUATIONS

Seven days after transplanting (DAT) the bacteria was applied to the soil in the minimum amount of $1x10^9$ UFC/mL. Four applications were performed at a dosage of 3 mL of the commercial product per liter of suspension per plant with the aid of a watering can. Subsequent applications were performed at intervals of 15 days.

Between the second (21 DAT) and the third (35 DAT) application, inoculation was performed with 5000 eggs and eventual juveniles (J2) of M. enterolobii in an interval of one week after and before the applications of the bacteria, respectively. The eggs and J2 were extracted from the roots according to the method of Hussey & Baker (1973) modified by Bonetti & Ferraz (1981). Inoculation was performed by pouring 1 mL of the suspension into four equidistant orifices, close to the collar of the plants.

From five days after the last application of the product with the bacteria and 26 DAI (days after inoculation), fortnightly evaluations were carried out with a portable infrared gas analyzer (Infrared Gas Analyzer – IRGA – model: LI-6800) of the variables: A = liquid photosynthesis (μmol m^{-2} s^{-1}); E = transpiration (mol m^{-2} s^{-1}), gs = stomatal conductance (mol m^{-2} s^{-1}) and USA = Water use efficiency (A/E-μmol m^{-2} s^{-1}. mol m^{-2} s^{-1}) in a fully expanded leaf located in the upper third of the plant and positioned between the 3rd and 5th pair of leaves from apex. All assessments were carried out between 7:30 am and 10:30 am until 118 DAI (146 DAT).

At 132 DAI the roots were washed in running water, weighed and stained with Phloxin B (Taylor & Sasser, 1978). The counting of galls and egg mass was carried out, according to the scale of notes established by Taylor & Sasser (1978). For calculation of gall index (GI) and egg mass index (IMO) (1: 1-2 galls or egg mass; 2: 3-10 galls or egg mass; 3: 11-30 galls or egg masses; 4: 31-100 galls or egg masses; and 5: over 100 galls or egg masses per root system). After counting, the nematodes were extracted from the roots following the method of Hussey & Barker (1973), modified by Bonetti & Ferraz (1981). The reproduction factor (FR) was obtained by the relation between final and initial population densities (FR=Pf/Pi) according to Oostenbrink (1966).

STATISTICAL ANALYSIS

The data were transformed by $\sqrt{x} + 1$ and submitted to analysis of variance. The Scott-Knott test (1974) was applied at 5% probability for grouping the means, using the statistical program RStudio.

RESULTS

PHYSIOLOGICAL VARIABLES

For transpiration at 26 and 44 DAI, the treatments that received the nematode and the bacteria, combined, showed a lower transpiration rate, differing from the other treatments (Table 1).

Regarding photosynthesis, the only difference observed was at 26 DAI, treatments `Pedro Sato´ and `Paluma´ inoculated and treated with B. met showed the lowest photosynthetic rates (Table 2).

For stomatal conductance, treatments with `Pedro Sato´ and `Paluma´ inoculated and treated, showed lower conductance at 26 DAI and 44 DAI (Table 3).

Transpiration (mol m^{-2} s^{-1})							
	26 DAI	**44 DAI**	**56 DAI**	**68 DAI**	**92 DAI**	**104 DAI**	**118 DAI**
T1	12.0 a	11.1 a	8.9 a	7.3 a	4.8 a	7.6 a	4.9 a
T2	12.6 a	11.6 a	10.6 a	9.1 a	4.6 a	8.0 a	4.3 a
T3	11.6 a	10.2 a	10.1 a	10. a	6.1 a	9.7 a	8.4 a
T4	11.4 a	11.0 a	8.8 a	7.4 a	5.1 a	9.0 a	5.5 a
T5	11.9 a	11.2 a	10.4 a	8.6 a	5.2 a	6.9 a	4.5a
T6	14.1 a	11.0 a	11.0 a	10.3 a	6.2 a	8.5 a	5.1 a
T7	9.10 b	6.6 b	7.5 a	8.8 a	4.8 a	6.8 a	6.2 a
T8	10.1 b	8.9 b	8.1 a	8.7 a	5.8 a	8.7 a	6.8 a
CV%	0.11	0.11	0.11	0.13	0.07	0.12	0.13

Table 1 – Transpiration rate of guava trees inoculated with *Meloidogyne enterolobii* and treated with *Bacillus methylotrophicus*, separately and associated

T1: `Pedro Sato´ uninoculated and untreated; T2: Uninoculated and untreated `Paluma´; T3: `Pedro Sato´ not inoculated and treated; T4: `Paluma´ not inoculated and treated; T5: 'Paluma' inoculated and untreated; T6: `Pedro Sato´ inoculated and not treated; T7: `Pedro Sato´ inoculated and treated and T8: `Paluma´ inoculated and treated; DAI: days after inoculation. DAT days after transplantation. Equal capital letters on the line do not show statistical difference. Equal lowercase letters in the column do not show statistical difference by the Scott-Knott test at 5% probability. (54, 85, 97, 110, 132 and 146 DAT).

Photosynthesis (µmol m^{-2} s^{-1})							
	26 DAI	44 DAI	56 DAI	68 DAI	92 DAI	104 DAI	118 DAI
T1	16.56 a	13.91 a	13.62 a	12.43 a	10.09 a	9.89 a	9.50 a
T2	14.72 a	12.88 a	12.90 a	14.21 a	8.55 a	9.43 a	6.50 a
T3	13.43 a	10.13 a	13.24 a	13.40 a	11.03 a	11.24 a	10.98 a
T4	14.18 a	11.49 a	13.16 a	13.19 a	10.08 a	9.96 a	8.39 a
T5	15.08 a	13.45 a	13.78 a	12.39 a	8.57 a	8.85 a	7.03 a
T6	16.10 a	13.08 a	13.83 a	14.87 a	11.54 a	10.22 a	9.04 a
T7	12.35 b	11.28 a	12.52 a	12.71 a	10.05 a	9.41 a	9.19 a
T8	12.91 b	11.80 a	12.90 a	13.00 a	10.54 a	9.83 a	9.37 a
CV%	9.32	8.27	8.19	12.00	11.55	12.00	14.48

Table 2 - Photosynthetic rate of guava trees inoculated with *Meloidogyne enterolobii* and treated with *Bacillus methylotrophicus,* separately and associated.

T1: `Pedro Sato´ uninoculated and untreated; T2: Uninoculated and untreated `Paluma´; T3: `Pedro Sato´ not inoculated and treated; T4: `Paluma´ not inoculated and treated; T5: 'Paluma' inoculated and untreated; T6: `Pedro Sato´ inoculated and not treated; T7: `Pedro Sato´ inoculated and treated and T8: `Paluma´ inoculated and treated; DAI: days after inoculation. Equal capital letters on the line do not show statistical difference. Equal lowercase letters in the column do not show statistical difference by the Scott-Knott test at 5% probability. (54, 85, 97, 110, 132 and 146 DAT).

Stomatal conductance (mol m^{-2} s^{-1})							
	26 DAI	**44 DAI**	**56 DAI**	**68 DAI**	**92 DAI**	**104 DAI**	**118 DAI**
T1	0.6210 a	0.5328 a	0.4688 a	0.4780 a	0.2336 a	0.3839 a	0.2458 a
T2	0.7242 a	0.6276 a	0.5735 a	0.5322 a	0.2217 a	0.4091 a	0.2014 a
T3	0.6382 a	0.5380 a	0.5493 a	0.5646 a	0.3380 a	0.5156 a	0.4724 a
T4	0.6272 a	0.5171 a	0.4526 a	0.3766 a	0.2572 a	0.4588 a	0.2821 a
T5	0.6451 a	0.5704 a	0.5709 a	0.5058 a	0.2967 a	0.3580 a	0.2019 a
T6	0.8205 a	0.6045 a	0.6090 a	0.6041 a	0.3739 a	0.4195 a	0.2536 a
T7	0.4533 b	0.3156 b	0.3603 a	0.4780 a	0.2337 a	0.3202 a	0.3001 a
T8	0.5377 b	0.4283 b	0.4526 a	0.4780 a	0.3272 a	0.4588 a	0.3794 a
CV%	4.61	4.81	4.79	6.20	5.00	6.00	6.68

Table 3 – Stomatal conductance of guava trees inoculated with *Meloidogyne enterolobii* and treated with *Bacillus methylotrophicus,* separately and associated.

T1: `Pedro Sato´ uninoculated and untreated; T2: Uninoculated and untreated `Paluma´; T3: `Pedro Sato´ not inoculated and treated; T4: `Paluma´ not inoculated and treated; T5: 'Paluma' inoculated and untreated; T6: `Pedro Sato´ inoculated and not treated; T7: `Pedro Sato´ inoculated and treated and T8: `Paluma´ inoculated and treated; DAI: days after inoculation. Equal capital letters on the line do not show statistical difference. Equal lowercase letters in the column do not show statistical difference by the Scott-Knott test at 5% probability. (54, 85, 97, 110, 132 and 146 DAT).

Efficiency in the use of water (µmol m^{-2} s^{-1}. mol m^{-2} s^{-1})							
	26 DAI	**44 DAI**	**56 DAI**	**68 DAI**	**92 DAI**	**104 DAI**	**118 DAI**
T1	1850 a	1737 a	1604 a	1712 a	2139 a	1316 a	2272 a
T2	1676 a	1423 a	1213 a	1587 a	1930 a	1186 a	1571 a
T3	1072 b	1019 b	1359 a	1392 a	1841 a	1192 a	1407 a
T4	1177 b	1224 b	1561 a	1843 a	1967 a	1134 a	1644 a
T5	1117 b	1114 b	1344 a	1476 a	1687 a	1309 a	1644 a
T6	1152 b	1256 b	1315 a	1475 a	1880 a	1199 a	1854 a
T7	1385 b	1365 b	1691 a	1503 a	2108 a	1627 a	1639 a
T8	1332 b	1238 b	1612 a	1501 a	1963 a	1166 a	1830 a
CV%	12.94	12.43	10.39	8.86	8.50	11.02	17.58

Table 4 - Efficiency in the use of water in guava trees inoculated with *Meloidogyne enterolobii* and treated with *Bacillus methylotrophicus,* separately and associated

T1: ` Pedro Sato´ non-inoculated and untreated; T2: Uninoculated and untreated `Paluma´; T3: `Pedro Sato´ not inoculated and treated; T4: `Paluma´ not inoculated and treated; T5: 'Paluma' inoculated and untreated; T6: `Pedro Sato´ inoculated and not treated; T7: `Pedro Sato´ inoculated and treated and T8: `Paluma´ inoculated and treated; DAI: days after inoculation. Equal capital letters on the line do not show statistical difference. Equal lowercase letters in the column do not show statistical difference by the Scott-Knott test at 5% probability. (54, 85, 97, 110, 132 and 146 DAT).

The treatments that received the isolated and combined nematode and bacteria showed a reduction in water use efficiency values at 26 and 44 DAI (Table 4).

NEMATOLOGICAL VARIABLES

There was no difference between treatments in root mass. Gall index, egg mass index and reproduction factor were higher in treatments that received nematodes regardless of the bacteria application (Table 5).

	IG	IMO	FR
T1	0 b	1 b	0 b
T2	0 b	0 b	0 b
T3	1 b	1 b	0 b
T4	1 b	2 b	0 b
T5	3 a	5 a	3 a
T6	4 a	5 a	2 a
T7	4 a	5 a	2 a
T8	5 a	5 a	3 a

Table 5 – Nematological variables in guava cv. Paluma and cv. Pedro Sato in soil treated or not treated with *Bacillus methylotrophicus* at 132 days after inoculation with 5000 eggs and eventual juveniles of *Meloidogyne enterolobii.*

DISCUSSION

Meloidogyne enterolobii and *B.met* isolates did not influence the stomatal conductance and transpiration of guava trees. Similar results were found by Melakeberhan et al. (1990) in the interaction: *M. incognita* and two grape cultivars, however *M. incognita* reduced stomatal conductance and transpiration of tomato (Khanna et al., 2019). *Meloidogyne exigua* and *M. paranaensis* reduced conductance and transpiration of coffee seedlings. *Meloidogyne enterolobii* did not influence the photosynthesis of guava trees, the same behavior was observed in coffee seedlings with *M. exigua*, However, different results were found in coffee plants inoculated with *M. paranaensis* (Goulart et al., 2019).

Plants infected with nematodes have a deficient root system, negatively influencing the absorption of water and nutrients essential to the physiological processes of the plant (Goulart et al., 2019). In the present study, *M. enterolobii* isolated did not influence the absorption of water by the roots and the transport of water and nutrients by the conducting vessels, due to the absence of changes in stomatal conductance, photosynthesis and transpiration, as a result of the low population density of the nematode. About 6000 eggs *M. incognita* were not enough to change the physiology of the cotton plant, and did not reduce the chlorophyll content (Lu et al., 2014). *Meloidogyne ethiopica* caused significant damage to tomato roots only 102 days after inoculation, consequently this damage reflected in physiological changes in the plant (Strajnar et al., 2012).

Studies indicate that rhizobacteria improve the photosynthetic rate indirectly, as they stimulate the production of the hormone cytokinin in the plant, which is associated with different mechanisms of the complex photosynthetic chain (Ahluwalia et al., 2021). The absence of alterations in stomatal conductance, photosynthesis and transpiration in the treatments that received only the bacteria may be a result of the form of application in the soil, and/or the concentration of the bacteria, or even the humidity of the soil, since it requires a minimum field capacity of 50% inoculation with rhizobacteria to increase positive responses in plant physiology (Castro et al., 2019).

Treatments they received *M. enterolobii* and *B.met* combined reduced stomatal conductance, water use efficiency, photosynthesis and transpiration. *Piriformospora indica* and *M. incognita* combined reduced photosynthesis in cucumber plants (Atia et al., 2020) *Meloidogyne incognita.*

Meloidogyne incognita e *Streptomyces spp.* combined reduced stomatal conductance and transpiration of tomato seedlings (Ma et al., 2017). The reduction in stomatal conductance caused by *M. enterolobii* in combination with *B.met* may have contributed to the reduction in photosynthesis because with closed stomata there was limitation in the absorption of CO_2. The release of water by the stomata to the atmosphere was also reduced with the closure of the stomata, decreasing transpiration in the treatments with *B. met* and *M. enterolobii,* combined.

The energy for the rhizobacteria to supply their needs comes from the photoassimilates of the plants. We can say that the guava tree photoassimilates may not have been sufficient to release energy for the rhizobacteria, since the guava tree was under stress induced by the presence of the nematode. This may have reflected in the bacteria's inability to positively influence the plant's physiological response, since under stress conditions, the bacteria performed poorly (Rampazzo et al., 2018).

Meloidogyne enterolobii and *B.met* isolated and associated, reduced the efficiency of water use, proving to be the most sensitive physiological variable to the attack of *M. enterolobii.* The reduction in water use efficiency induced by the nematode cannot be explained by changes in stomatal conductance, photosynthesis and transpiration rate, since there were no variations in these parameters when treatments received only the nematode, and thus the change in water efficiency Water utilization is explained by non-stomatal factors such as Rubisco activity, maximum Rubisco carboxylation rate, Ribulose regeneration capacity mediated by maximum electron transport rate, and triose phosphate utilization rate (Lu et al., 2014; Jiao et al., 2017).

Similar results were found by Atia et al. (2020) in which *M. incognita* alone and in combination with *Piriformospora indica* reduced water use efficiency in cucumber. The amount of water available to the plants, vapor pressure, leaf and ambient temperature, nitrogen available to the plants are factors that above or below the required limits may have contributed to the reduction in the use of water and were potentiated by the nematode and the bacteria alone and in combination (Hatfield & Dold, 2019; Atia et al., 2020).

No physiological variable evaluated has changed after 56 DAI (84 DAT). The guava tree is a rustic plant that adapts to different environmental conditions (Silva et al., 2010), and the plant in an advanced stage of development may not suffer so much from nematode infection due to the greater energy support to tolerate the attack without reflect negatively on their physiological performance (Melakeberhan et al., 1990). Furthermore, in subsequent stages of development the fruit tree may be able to develop tolerance mechanisms that preclude changes in stomatal conductance, photosynthesis and transpiration.

It can be stated that under the conditions of the experiment, *B. met* did not trigger antagonistic mechanisms on *M. enterolobii,* since statistical differences observed in nematological variables were between inoculated and non-inoculated treatments. There was no difference between root masses. The gall index, egg mass index and reproduction factor were higher in treatments that received nematodes, regardless of the application of the bacteria. In some guava roots that did not receive nematodes, small galls were noticed, but eggs were not recovered.

The bacteria did not contribute to reducing the number of galls and egg masses. *Pseudomonas aeruginosa* and *Burkholderia gladioli* separately and associated with *M. incognita* also did not influence tomato root mass, but reduced the number of galls

(Khanna et al., 2019). Of 27 isolates of plant growth-promoting bacteria, five led to an increase in the number of plant galls. *M. incognita* (Viljoen & Labuschagne, 2019). *Bacillus subtilis* e *B. pumilus* reduced *M. incognita* in cowpea (Abd-El-Khair et al., 2019). Zhou et al. (2016) observed for the first time the nematicidal capacity of *B. met* about *M. incognita*, however, the strain used in the present assay did not show nematicidal activity in the control of *M. enterolobii*. *Meloidogyne incognita* and *P. fluorescens* in simultaneous inoculations did not efficiently reduce the number of egg mass in tomato root, however, the reduction was greater when the inoculation scheme was *P. fluorescens* and after 15 days inoculated: *M. incognita* (Noureldeen et al., 2021). The reproduction factor was higher in tomato plants inoculated with *M. javanica* followed by inoculation with *Paecilomyces lilacinus* with an interval of 10 days compared the simultaneous inoculations of the organisms and the inoculation of *P. lilacinus* 10 days before inoculation with *M. javanica* (Ganaie & Khan, 2010).

The mode and scheme of the applications of the bacteria in time, the concentration and genotypes of the nematode and bacteria may have contributed to the inefficiency of the bacteria in the control of *M. enterolobii* (Zhou et al., 2016).

CONCLUSIONS

Meloidogyne enterolobii and *Bacillus methylotrophicus* combined reduced stomatal conductance, photosynthesis and transpiration of guava trees during the initial phase of fruit development. *Meloidogyne enterolobii* and *B. met* isolated and combined reduced water use efficiency at 26 and 44 DAI. After 44 DAI, there was no change in the evaluated physiological parameters. There was no difference in root mass. The nematological parameters were not influenced by the bacteria, since the difference observed was between inoculated and non-inoculated treatments.

REFERENCES

ABD-EL-KHAIR, H.; EL-NAGDI, W. M. A.; YOUSSEF, M.M.A.; ABD ELGAWAD, M. M.; DAWOOD, M. G. 2019. Protective effect of *Bacillus subtilis*, *B. pumilus*, and *Pseudomonas fluorescens* isolates against root knot nematode *Meloidogyne incognita* on cowpea. **Bulletin of the National Research Centre**, v. 43, p. 1–7, 2019.

ABRÃO, M. M.; MAZZAFERA, P. Efeitos do Nível de inóculo de *Meloidogyne incognita* em algodoeiro. **Bragantia**, v. 60, p. 19–26, 2001.

AHLUWALIA, O.; SINGH, P. C.; BHATIA, R. Resources , environment and sustainability, a review on drought stress in plants : implications , mitigation and the role of plant growth promoting rhizobacteria. **Resources, Environment and Sustainability**, v. 5, p. 1-13, 2021.

ATIA, M. A. M.; ABDELDAYM, E. A.; ABDELSATTARM.; IBRAHIM, D. S. S.; IBRAHIM, S.; ELWAHAB, M. A.; OSMAN, G. H.; ARIF, I. A.; ABDELAZIZ, M. E. *Piriformospora indica* promotes cucumber tolerance against root-knot nematode by modulating photosynthesis and innate responsive genes. **Saudi Journal of Biological Sciences**, v. 27, p. 279–287, 2020.

AZAM, T.; HISAMUDDIN.; SINGH, S.; ROBAB, M.I. Effect of different inoculum levels of *Meloidogyne incognita* on growth and yield of *Lycopersicon esculentum* , and internal structure of infected root. **Archives Of Phytopathology And Plant Protection**, v. 44, p. 1829–1839, 2011.

BONETTI, J.I.; FERRAZ, S. Modificações do método de Hussey& Barker para extração de ovos de *Meloidogyne exigua* em raízes de cafeeiro. **Fitopatologia Brasileira**, v. 6, p. 553, 1981.

CARNEIRO, R.; ALMEIDA, M. R. A. Técnica de eletroforese usada no estudo de enzimas dos nematóides de galhas para identificação de espécies. **Nematologia Brasileira**, v. 25, p. 35–44, 2001.

CASTRO, G. L. S.; SILVA JÚNIOR, D. D.; VIANA, R. G.; RÊGO, M. C. F.; SILVA, G. B. Photosynthetic apparatus protection and drought effect mitigation in açaí palm seedlings by rhizobacteria. **Acta Physiologiae Plantarum**, v. 419, p. 1–12, 2019.

COLLETT, R. L.; MARAIS, M.; DANEEL, M.; RASHIDIFARD, M.; FOURIE, H. *Meloidogyne enterolobii,* a threat to crop production with particular reference to sub-Saharan Africa: an extensive, critical and updated review. **Nematology**, v. 0, p. 1–39, 2021.

DALASTRA, G. M.; ECHER, M. D. M.; GUIMARÃES, V. F.; HACHMANN, T. L.; INAGAKI, A. M. Trocas gasosas e produtividade de três cultivares de meloeiro conduzidas com um e dois frutos por planta. **Bragantia**, v. 73, p. 365–371, 2014.

DOMICIANO, G. P.; RESENDE, R. S.; RODRIGUES, F. A. Alteração na fotossíntese de plantas infectadas por patógenos. **Revista Anual Patolologia de Plantas**, v. 17, p. 305-339, 2009.

Eppo, 2023. EPPO. *Meloidogyne enterolobii(MELGMY)*. Disponível em: <*https://gd.eppo.int/taxon/MELGMY/distribution*>.

GANAIE, M. A.; KHAN, T. A. Biological potential of *Paecilomyces lilacinus* on pathogenesis of *Meloidogyne javanica* infecting tomato plant. **European Journal of Applied Sciences**, v. 2, p. 80–84, 2010.

GOULART, R. R.; TERRA, W. C.; SALGADO, S. M. L.; ALVES, J. D.; CAMPOS, V. P.; FATOBENE, B. J. R.; MARCHIORI, P. E. R.; SOUZA, S. R.; OLIVEIRA, R. D. A. L. *Meloidogyne paranaensis* and *M. exigua* alter coffee physiology. **Nematology**, v. 21, p. 459–467, 2019.

HATFIELD, J. L.; DOLD, C. Water-use efficiency:advances and challenges in a changing climate. **Frontiers in Plant Science**, v. 10, p. 1–14, 2019.

HUSSEY, R. S.; BAKER, K. R. A. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. **Plant Disease**, v. 57, p. 1025–1028, 1973.

JIAO, L.; WANG, L.; ZHOU, Q.; HUANG, X. Ecotoxicology and environmental safety stomatal and non-stomatal factors regulated the photosynthesis of soybean seedlings in the present of exogenous bisphenol A. **Ecotoxicology and Environmental Safety**, v. 145, p. 150–160, 2017.

KHANNA, K.; SHARMA, A.; OHRI, P.; BHARDWAJ, R.; ABD-ALLAH, E. F.; HASHEM, A.; AHMAD, P. Impact of plant growth promoting rhizobacteria in the orchestration of *Lycopersicon esculentum* Mill. Resistance to plant parasitic nematodes: A metabolomic approach to evaluate defense responses under field conditions. **Biomolecules**, v. 9, p. 1-30, 2019.

KIEWNICK, S.; DESSIMOZ, M.; FRANCK, L. Effects of the Mi-1 and the N root-knot nematode-resistance gene on infection and reproduction of *Meloidogyne enterolobii* on tomato and pepper cultivars. **Journal of Nematology**, v. 41, p. 134–139, 2009.

LI, X.; YANG, D.; NIU, J.; ZHAO, J.; JIAN, H. De novo analysis of the transcriptome of *meloidogyne enterolobii* to uncover potential target genes for biological control. **International Journal of Molecular Sciences**, v. 17, p. 1–14, 2016.

LU, P.; DAVIS, R. F.; KEMERAIT, R. C.; VAN IERSE, M. W.; SCHERM, H. 2014. Physiological effects of *Meloidogyne incognita* infection on cotton genotypes with differing levels of resistance in the greenhouse. **Journal of Nematology**, v. 46, p. 352–359, 2014.

MA, Y.; LI, Y.; LAI, H.; GUO, Q.; XUE, Q. Effects of two strains of *Streptomyce*s on root-zone microbes and nematodes for biocontrol of root-knot nematode disease in tomato. **Applied Soil Ecology**, v. 112, p. 34–41, 2017.

MELAKEBERHAN, H.; FERRIS, H.; DIAS, J. M. Physiological response of resistant and susceptible *Vitis vinifiera* cultivars to *Meloidogyne incognita*. **Journal of Nematology**, v. 22, p. 224–230, 1990.

MIORANZA, T. M.; INAGAKI, A. M.; MÜLLER, M. A.; STANGARLIN, J. R.; GUIMARÃES, V. F.; KLEIN, J.; KUHN, O. J. Gas exchange and photosynthetic light response curves in nematode-infected tomato plants treated with *Thuya occidentalis*. **Australian Journal of Crop Science**, v. 12, p. 583–591, 2018.

NOURELDEEN, A.; ASIF, M.; ANSARI, T.; KHAN, F.; SHARIQ, M.; AHMAD, F.; MFARREJ, M. F. B.; KHAN, A.; TARIQ, M.; SIDDIQUI, M. A.; AL-BARTY, A.; DARWISH, H. Effect of individual , simultaneous and sequential inoculation of *Pseudomonas fluorescens* and *Meloidogyne incognita* on growth, biochemical, enzymatic and nonenzymatic antioxidants of tomato (*Solanum lycopersicum* L.). **Plants**, v. 10, p. 1–15, 2021.

OLIVEIRA, I. P.; OLIVEIRA, L. C.; MOURA, C. S. F. T.; LIMA JÚNIOR, A. F.; ROSA, S. R. A. Cultivo da goiabeira: do plantio ao manejo. **Revista Faculdade Montes Belos**, v. 5, p. 137–156, 2012.

OOSTENBRINK, M. 1966. Major characteristics of the relation between nematodes and plants. **Mededelingen / Landbouwhogeschool Wageningen**, v. 66, p. 1–46, 1966.

PEREIRA, F. M.; NACHTIGAL, J. C. Melhoramento da goiabeira. *Melhoramento de Fruteiras Tropicais*. Editora: UFV, Viçosa, p.78, 2002.

PEREIRA, F. O. M.; SOUZA, R. M.; SOUZA, P. M.; DOLINSKI, C.; SANTOS, G. K. Estimativa do impacto econômico e social direto de *Meloidogyne mayaguensis* na cultura da goiaba no Brasil. **Nematologia Brasileira**, v. 33, p. 176–181, 2009.

RAMPAZZO, P. E.; MARCOS, F. C. C.; CIPRIANO, M. A. P.; MARCHIOR, P. E. R.; FREITAS, S. S.; MACHADO, E. C.; NASCIMENTO, L. C.; BROCCHI, M.; RIBEIRO, R. V. Rhizobacteria improve sugarcane growth and photosynthesis under well-watered conditions. **Annals of Applied Biology**, v. 172, p. 309-320, 2018.

SILVA, L. S.; MENDES, A. M. S.; OLIVEIRA, A. R.; PARANHOS, B. A. J.; SANTOS, C. A. F.; SILVA, D. J.; BASTOS, D. C.; BATISTA, D. C.; BARBOSA, F. R. **A cultura da goiaba**. 2.ed. Embrapa Informação Tecnológica-Brasília, p. 1-180, 2010.

STRAJNAR, P.; ŠIRCA, S.; UREK, G.; ŠIRCELJ, H.; ŽELEZNIK, P.; VODNIK, D. Effect of *Meloidogyne ethiopica* parasitism on water management and physiological stress in tomato. **European Journal of Plant Pathology**, v. 132, p. 49–57, 2012.

TAYLOR, A. L.; SASSER, J. N. **Biology, identification and control of root-knot nematodes (*Meloidogyne* species)**. Dep. of Plant Pathology, North Carolina State University, 111p, 1978.

TIGANO, M.; SIQUEIRA, K.; CASTAGNONE-SERENO, P.; MULET, K.; QUEIROZ, P.; SANTOS, M.; TEIXEIRA, C.; ALMEIDA, M.; SILVA, J.; CARNEIRO, R. Genetic diversity of the root-knot nematode *Meloidogyne enterolobii* and development of a SCAR marker for this guava-damaging species. **Plant Pathology**, v. 59, p. 1054-1061, 2010.

VILJOEN, J. J. F.; LABUSCHAGNE, N. Biological control of the root-knot nematode *Meloidogyne incognita* on tomatoes and carrots by plant growth-promoting rhizobacteria. **Tropical Plant Pathology**, v. 44, p. 284–291, 2019.

ZHOU, L. YUEN, G.; WANG, Y.; WEI, L.; JI, G. Evaluation of bacterial biological control agents for control of root- knot nematode disease on tomato. **Crop Protection**, v. 84, p. 8–13, 2016.