Universidade Federal Rural do Semi-Árido
Pró-Reitoria de Pesquisa e Pós-Graduação
https://periodicos.ufersa.edu.br/index.php/caatinga

ISSN 0100-316X (impresso)
ISSN 1983-2125 (online)
http://dx.doi.org/10.1590/1983-21252023v36n201rc

Cover crops and biocontrol agents in the management of nematodes in soybean crop

Plantas de cobertura e agentes de biocontrole no manejo de nematoides na cultura da soja

Fernando G. de Araujo[1]*, Samuel J. C. Teixeira[2], Jean C. de Souza[1], Claudia R. D. Arieira[3]

[1]Laboratory of Integrated Management of Nematodes, Instituto Federal Goiano, Urutaí, GO, Brazil. [2]Agronomy projects coordination (Technical Marketing), Lallemand Soluções Biológicas, Patos de Minas, MG, Brazil. [3]Departament of Agronomic Sciences, Universidade Estadual de Maringá, Umuarama, PR, Brazil.

ABSTRACT - In the Brazilian Cerrado, phytonematodes, phytonematodes are a challenge for agriculture, and the association of forms of control is the best strategy to be used in coexistence with this pathogen. Faced with this problem, this study aimed to evaluate the efficiency of cover crops and biocontrol agents in the management of nematodes in soybean. For each nematode species under study (*Meloidogyne incognita* and *Pratylenchus brachyurus*), two experiments were carried out under greenhouse conditions in a completely randomized design arranged in a 6 x 2 factorial scheme with eight replications. Six cover crops (millet ADRG 9050, millet ADR 300, *Urochloa ruziziensis*, *Crotalaria ochroleuca*, *C. spectabilis,* and corn hybrid DKB 290) and two treatments (treated or not with *Bacillus subtilis* and *B. methylotrophicus* - 1x10^6 CFU per seed) were evaluated. The cover crops were sown in soil infested with nematodes and then soybeans were sown under the straw, and cultivated for 45 and 60 days, for *M. incognita* and *P. brachyurus*, respectively. Fresh root mass, total nematode population, and the number of nematodes per gram of root were evaluated. The association of microorganisms with *C. spectabilis*, *U. ruziziensis,* and millet ADR 300 conferred an additional effect in reducing the population of *P. brachyurus*. For *M. incognita,* the association was successful only for combining *C. spectabilis* with *B. methylotrophicus*. DKB 290 corn, when treated with *B. methylotrophicus*, had the nematode population per gram of soybean root reduced by 90% compared to plants that did not receive biological treatment.

Keywords: *Meloidogyne incognita*. *Pratylenchus brachyurus*. Biological control.

RESUMO - No Cerrado brasileiro, os fitonematoides são um desafio para agricultura, sendo a associação de formas de controle a melhor estratégia a ser utilizada na convivência com esse patógeno. Diante dessa problemática, o objetivo desse trabalho foi avaliar a eficiência de plantas de cobertura e agentes de biocontrole no manejo de nematoides na cultura da soja. Foram desenvolvidos dois ensaios em condições de casa de vegetação para os nematoides *Meloidogyne incognita* e *Pratylenchus brachyurus*, em delineamento inteiramente casualizado, em esquema fatorial 6 (plantas de cobertura: milheto ADRG 9050, milheto ADR 300, *Urochloa ruziziensis, Crotalaria ochroleuca, C. spectabilis* e milho DKB 290) x 2 (tratados ou não com *Bacillus subtilis* e *B. methylotrophicus* - 1x10^6 UFC por semente), com oito repetições. As plantas de cobertura foram semeadas em solo infestado com nematoides e na sequência semeada soja sob a palha, e cultivada por 45 e 60 dias, para *M. incognita* e *P. brachyurus*, respectivamente. Foram avaliadas a massa fresca de raízes e o número de nematoides totais e por grama de raiz. A associação dos microrganismos com *C. spectabilis, U. ruziziensis* e milheto ADR 300 conferiram efeito adicional na redução da população de *P. brachyurus*. Para a espécie *M. incognita* a associação foi bem sucedida apenas para a combinação da *C. spectabilis* com *B. methylotrophicus*. O milho DKB 290, quando tratado com *B. methylotrophicus*, teve a população do nematoide por grama de raiz da soja reduzida em 90 % em comparação às plantas que não receberam o tratamento biológico.

Palavras-chave: *Meloidogyne incognita*. *Pratylenchus brachyurus*. Controle biológico.

Conflict of interest: The authors declare no conflict of interest related to the publication of this manuscript.

Received for publication in: June 7, 2021.
Accepted in: October 13, 2022.

***Corresponding author:**
<fernando.godinho@ifgoiano.edu.br>

INTRODUCTION

In the Brazilian Cerrado, several genera of nematodes are associated with cropping systems, especially the soybean cyst nematode - SCN (*Heterodera glycines*), root-lesion nematodes (*Pratylenchus brachyurus* and *P. zeae*), root-knot nematodes (*Meloidogyne incognita* and *M. javanica*), reniform nematode (*Rotylenchulus reniformis*) and spiral nematode (*Helicotylenchus dihystera* and *Scutellonema brachyurus*) (DIAS et al., 2010). Among these genera, the root lesion nematode has great expression due to its wide distribution and a high degree of polyphagy.

P. brachyurus is a migratory endoparasite that moves inside the roots, causing mechanical damage and releasing toxins and enzymes in the root cortex, both during penetration and movement, causing the destruction of the root system and, as a result, they are known as root lesion nematode. They rank second in terms of global and national economic damage and impacts. Losses can reach up to 50% in soybean yield (GOULART, 2008). However, in soybean, losses have increased dramatically in recent seasons, reaching 50% in Cerrado soils

(FRANCHINI et al., 2014). This is because the nematode has been benefiting from changes in the production system and the incorporation of areas with sandy textured soils, further increasing the vulnerability of the crop.

Controlling nematodes is a difficult task. Generally, the producer needs to live with the pathogen through the management of population levels in the soil. Control methods against nematodes have relative efficiency because they have a low permeable cuticle, which gives them great resistance to physical and chemical agents (AlCANFOR et al., 2001). Succession with resistant crops is the primary tool for suppressing this bridge of survival between crops. Without a susceptible host, the pathogen dies without food or completing its life cycle, thus acting as a control by relative eradication of the initial inoculum, preventing damage to the main crop (JOHNSON, 1985).

Biological control has shown promising results in keeping populations low and reducing the economic damage caused by the pathogen. Among the potential agents for biological control, bacteria of the genus *Bacillus* stand out. Although they do not directly parasitize nematodes, species of the *Bacillus* genus have multiple forms of action on these pathogens. *Bacillus* spp. can act on nematodes by antibiosis; that is, the bacterium produces substances that inhibit the hatching of eggs and the migration of nematodes in the soil for food. Another mode of action is competition, in which the bacterium colonizes the root and forms a physical and chemical barrier, preventing or hindering the nematode from entering the root system. In addition, when they are associated with the roots or in their surroundings (rhizosphere), *Bacillus* release chemicals that mimic this region and make it difficult for the nematode to locate the root system (ARIEIRA, 2020).

The association of *Bacillus* species has shown promising effects when used together with cover crops, especially those that do not resist all nematode species (SILVA, 2016). Costa, Pasqualli and Prevedello (2014) found that *C. spectabilis*, even not treated with biocontrol agents, contributes significantly to reducing the *P. brachyurus* population. In addition to this contribution, the results show that *C. spectabilis*, when treated with *B. subtilis*, reduced the total nematode population and the population per gram of root by 88% and 80%, respectively. In this context, the present study aimed to evaluate the efficiency of cover crops and the species of *Bacillus subtilis* and *B. methylotrophicus* in the management of the nematodes *P. brachyurus* and *M. incognita* in the soybean crop.

MATERIAL AND METHODS

In a completely randomized design, four experiments were carried out under greenhouse conditions at the State University of Maringá, two with *P. brachyurus* and two with *M. incognita*. The experiments with *P. brachyurus* were arranged in a 6 x 2 factorial scheme (six cover crops, with and without the application of biological), with ten replications, and the experiments with *M. incognita* in a 5 x 2 factorial scheme (cover crops, with and without the application of biological), with eight replications (Table 1). In the first experiment, for both nematodes, biological treatment was used with *B. methylotrophicus* UFPEDA 20 and, in the second, *B. subtilis* UFPEDA 764 as a biocontrol agent (Table 1).

Initially, soybean seeds of the cultivar BRASMAX DESAFIO RR were deposited in pots with 2 L of soil and sand (proportion 1:1) autoclaved for 2 hours at 120 ^{o}C. Ten days after sowing, the plants were inoculated with 500 specimens of *P. brachyurus* or 4,000 eggs and eventual second-stage juveniles (J2) of *M. incognita*. Inoculums were obtained from pure populations of nematodes, kept in soybean, in a greenhouse for two months, and extracted from the roots by the extraction process proposed by Hussey and Barker (1973) and adapted by Boneti and Ferraz (1981). This study step aimed to enable the prior multiplication of the nematode in the same container where the cover crops were sown, simulating a soil with cultural remains of soybean roots parasitized by the nematode.

After 45 days of inoculation for the experiments with *P. brachyurus* and 50 days for the experiments with *M. incognita*, the shoot of the soybean plants was discarded, the soil was lightly turned, and then, the treatments were introduced, which consisted of the plants: *Crotalaria ochroleuca*, *C. spectabilis*, *Urochloa ruziziensis* (=*Brachiaria ruziziensis*), millet cv. ADRG 9050, millet ADR 300, and corn DKB 290, with or without seed treatment, with the products *B. methylotrophicus* in the first and *B. subtilis* in the second. For the experiments with *M. incognita*, *C. ochroleuca* was not used. For *C. ochroleuca* and *B. ruziziensis*, two seeds were deposited per pot, while for the other species, only one seed per pot. For seed treatment, an amount of 1×10^6 propagules of *Bacillus* sp. per seed was used. The product volume was deposited on the seeds, regardless of the number of seeds per pot, being the concentrations of the commercial products *B. methylotrophicus* (1×10^9) and *B. subtilis* (3×10^9).

The cover crops were cultivated for 70 days, and the shoot was cut and placed on the ground. Subsequently, each pot received a soybean seed, cultivated for 70 days for the experiments with *P. brachyurus* and 60 days with *M. incognita*. Then, the plants were collected, separating shoots and roots. The roots were carefully washed, weighed, and subjected to nematode extraction according to the methodology proposed by Coolen and D'Herde (1972). In a Peter Chamber, under an optical microscope, the total population of nematodes was determined, and the number of nematodes per gram of root was calculated.

The nematological data were submitted to the analysis of Generalized Linear Models (GLM), following the *Poisson* distribution and the means compared by the Tukey test for GLM at 5% probability. Generalized Linear Models (GLM) were used for the biometric data, following the Gamma distribution and the means compared by the Tukey test for GLM at 5% probability.

Table 1. Description of treatments with the cover crops treated and not treated with the microorganisms *Bacillus* methylotrophicus (Experiments 1 and 3) and *B. subtilis* (Experiments 2 and 4), and their concentrations per seed, in the studies with *Pratylenchus brachyurus* and *Meloidogyne incognita*.

Experiments with *Pratylenchus brachyurus*				
Treatments	Cover crops	Experiment 1	Experiment 2	U.F.C/seed
T1	Corn DKB 290	-	-	-
T2	Millet ADRG 9050	-	-	-
T3	Millet ADR 300	-	-	-
T4	*U. ruziziensis*	-	-	-
T5	*C. ochroleuca*	-	-	-
T6	*C. spectabilis*	-	-	-
T7	Corn DKB 290	*B. methylotrophicus*	*B. subtilis*	$1x10^6$
T8	Millet ADRG 9050	*B. methylotrophicus*	*B. subtilis*	$1x10^6$
T9	Millet ADR 300	*B. methylotrophicus*	*B. subtilis*	$1x10^6$
T10	*U. ruziziensis*	*B. methylotrophicus*	*B. subtilis*	$1x10^6$
T11	*C. ochroleuca*	*B. methylotrophicus*	*B. subtilis*	$1x10^6$
T12	*C. spectabilis*	*B. methylotrophicus*	*B. subtilis*	$1x10^6$
Experiments with *Meloidogyne incognita*				
Treatments	Cover plant	Experiment 1	Experiment 2	U.F.C/seed
T1	Corn DKB 290	-	-	-
T2	Millet ADRG 9050	-	-	-
T3	Millet ADR 300	-	-	-
T4	*U. ruziziensis*	-	-	-
T5	*C. spectabilis*	-	-	-
T6	Corn DKB 290	*B. methylotrophicus*	*B. subtilis*	$1x10^6$
T7	Millet ADRG 9050	*B. methylotrophicus*	*B. subtilis*	$1x10^6$
T8	Millet ADR 300	*B. methylotrophicus*	*B. subtilis*	$1x10^6$
T9	*U. ruziziensis*	*B. methylotrophicus*	*B. subtilis*	$1x10^6$
T10	*C. spectabilis*	*B. methylotrophicus*	*B. subtilis*	$1x10^6$

RESULTS AND DISCUSSION

There was a significant interaction of the treatment of cover crops seeds with the product *B. methylotrophicus* for the number of specimens of *P. brachyurus* per gram of root and the total number of specimens. All treatments with cover crops treated with *Bacillus methylotrophicus* promoted a reduction in the total number of nematodes in soybean cultivated in sequence, compared to corn, with the treatments being more efficient in controlling the nematode: *C. ochroleuca*, followed by millet ADR 300, *C. spectabilis*, *U. ruziziensis,* and millet ADRG 9050 (Table 2).

Although the multiplication of *P. brachyurus* under the effect of *U. ruziziensis* has already been elucidated by Silva et al. (2013), in this study, we observed that when we treated *U. ruziziensis* seeds with *B. methylotrophicus*, there was a reduction of 53% and 67% in the total nematode population and per gram of root, respectively.

In the treatment without *B. methylotrophicus*, the greatest reductions of *P. brachyurus* were promoted by the treatments *C. spectabilis*, *C. ochroleuca*, millet ADR300, and *U. ruziziensis*. Results corroborate with Cruz, Asmus and Garcia (2020), where reductions in *P. brachyurus* populations are evidenced only by the effect of using sunn hemp in succession.

The nematode population per gram of root in soybean cultivated after ADRG9050 millet was higher than that observed in corn without biological application (Table 2). These data indicate that, except millet ADRG9050, the cover crops themselves efficiently reduce the population of *P. brachyurus*. However, when the effect of the *B. methylotrophicus* treatment was studied within each cover, it was possible to observe the additional gain of the integrated management associating cover crops with biological control. The exceptions were *C. spectabilis* and corn, for which the association with *B. methylotrophicus* had no positive effect on reducing total nematode populations.

The results of the present study corroborate with Silva et al. (2018). The authors observed that soybean rotation with *C. ochroleuca* led to a reduction in *P. brachyurus population* density. In this context, it is observed that *C. ochroleuca* can reduce the population of *P. brachyurus*, but the association with *B. methylotrophicus* increases the effect of the legume in reducing the population of the nematode.

Table 2. *Pratylenchus brachyurus* total and per gram of soybean root after cultivation of cover crops, whose seeds were treated or not with *Bacillus methylotrophicus* and percentage of nematode reduction within each cover crop, at 70 days after sowing.

Treatments	Total nematode		
	Without *B. methylotrophicus*	With *B. methylotrophicus*	% Reduction
Corn	28397 aB	36061 aA	-27
Millet ADRG 9050	19772 bA	19261 bB	35
Millet ADR 300	13217 dA	4120 eB	69
U. ruziziensis	17372 cA	8098 cB	53
C. ochroleuca	7622 eA	2923 fB	62
C. spectabilis	3778 fB	4446 dA	-18
	Nematode per gram of root		
Corn	12860 bA	13303 aA	-3
Millet ADRG 9050	14710 aA	10681 fB	27
Millet ADR 300	7465 dA	2462 dB	67
U. ruziziensis	10931 cA	3637 cB	67
C. ochroleuca	2514 eA	1121 fB	55
C. spectabilis	1575 fA	1328 eB	16
p-value	$<2.2 \times 10^{-16}$		

Means followed by the same lowercase letter in the column and uppercase letter in the line do not differ by the Tukey test at 5% probability. p = probability of error.

Analyzing the number of nematodes per gram of root, the results were significant since, except for corn, for which the association with *B. methylotrophicus* did not differ from untreated plants, the other treatments were efficient in controlling the nematode (Table 2). For this variable, the reductions promoted by *B. methylotrophicus* were 67% when associated with millet ADR300 and *U. ruziziensis* and 55% when the bacterium was applied with *C. ochroleuca*. The use of *B. methylotrophicus* with millet ADRG9050 and *C. spectabilis* reduced the nematode population by 27% and 16%, respectively.

The fresh mass of roots of soybean plants only differed according to the different cover crops used, regardless of the biological treatment. The highest fresh root masses were observed in soybeans cultivated in succession to corn and the two *Crotalaria* species (Table 3). In addition to these species, corn also promoted an increase in the root mass of the subsequent soybean.

Table 3. Fresh mass of shoot and root of soybean infected by *Pratylenchus brachyurus* after cultivation of cover crops, whose seeds were treated or not treated with *Bacillus methylotrophicus* regardless of the treatment.

Treatments	Root fresh mass (g)
Corn DKB290	2.74 a
Millet ADRG 9050	1.87 b
Millet ADR 300	1.98 b
U. ruziziensis	2.37 b
C. ochroleuca	3.27 a
C. spectabilis	3.17 a
CV (%)	37.46

Means followed by the same letter in the column do not differ by the Tukey test at 5% probability. CV= coefficient of variation.

From the results obtained in the experiments with *M. incognita*, it was verified that in susceptible plants, such as corn, the treatment with *B. methylotrophicus* was quite efficient, reducing the multiplication of the root-knot nematode by 91.3% (Table 4). In addition to the treatment with corn, the treatment with *B. methylotrophicus* also promoted an additive effect (33.9%) in reducing nematodes in soybean cultivated after *C. spectabilis*.

Table 4. *Meloidogyne incognita* total and per gram of soybean root after cultivation of cover crops, whose seeds were treated or not with *Bacillus methylotrophicus* and percentage of nematode reduction within each cover crop, at 60 days after sowing.

Treatments	With *B. methylotrophicus*	Without *B. methylotrophicus*	With *B. methylotrophicus*	Without *B. methylotrophicus*	% Reduction by *B. methylotrophicus*
	Total nematode		% Reduction of coverage concerning the control		
Corn DKB 290	1262 eA	14521 aB	-	-	91.3
Millet ADRG 9050	1654 dB	1206 dA	-	91.7	-
Millet ADR 300	8527 aB	785 eA	-	94.6	-
U. ruziziensis	2527 cB	2236 cA	-	84.6	-
C. spectabilis	4844 bA	7331bB	-	49.5	33.9
p-value	$2.2e^{-16}$				
Treatments	Nematode/g of root		% Reduction of coverage concerning the control		% Reduction by *B. methylotrophicus*
Corn	88 eA	1301 bB	-	-	93.2
Millet ADRG 9050	180 dA	194 dA	-	85.1	7.0
Millet ADR 300	518 aB	117 eA	-	91.0	-
U. ruziziensis	287 cA	381 cB	-	70.1	24.7
C. spectabilis	367 bA	1765 aB	-	-	79.2
p-value	$2.2e^{-16}$				

Means followed by the same lowercase letter in the column and uppercase letter in the line do not differ by the Tukey test at 5% probability. p = probability of error.

In general, soybean cultivated after cover crops treated with *B. methylotrophicus* had the highest number of total nematodes (Table 4). On the other hand, evaluating the number of nematodes per gram of root, it was observed that *B. methylotrophicus* promoted reductions in a population density ranging from 7.0% (for soybean cultivated after millet ADR9050) to 93.2% (for soybean grown after corn). Such reductions were 24.7 and 79.2% for soybean cultivation after *U. ruziziensis* and *C. spectabilis* when treated with *B. methylotrophicus*. The gain in root volume explains that the action of *B. methylotrophicus* can be better visualized in the analysis of nematodes per gram of root since many rhizobacteria are considered plant growth promoters.

The cover crops individually controlled the nematode, with reductions in total nematode numbers that ranged from 49.5% to 94.6% and in the numbers of nematodes per gram of root, whose reductions ranged from 70.1% to 91 .0%. Likewise, *B. methylotrophicus* applied to the susceptible plant (corn DKB 290) also efficiently controlled *M. incognita*. The interaction between cover crops and biological control agents is complex and needs better elucidation.

In plants treated with *B. subtilis*, it was observed that except for *U. ruziziensis*, whose final nematode population in soybean was higher than that found in corn, there was a reduction in the reproduction of *P. brachyurus* for the succession with the other coverings, *C. spectabilis* being the most efficient in reducing the nematode, followed by *C. ochroleuca*, millet ADR 300 and millet ADRG 9050 (Table 5). *Crotalaria* species contribute to reducing soil nematodes through different mechanisms, such as non-host or alternative host behavior; production of toxic or inhibitory allelochemicals; stimulation of antagonistic flora and fauna; entrapment of the nematode in the root, inhibiting its multiplication (WANG; SIPES; SCHMITT, 2002).

There was a significant interaction between the treatment of cover crops seeds with *B. subtilis* for the total number of nematodes. All cover crops that did not receive *B. subtilis* efficiently reduced the nematode in soybeans planted in succession (Table 5). Still, for the total number of nematodes, evaluating the effect of *B. subtilis* within each plant, it was found high efficiency of the bacterium in promoting nematode control, with the greatest reductions observed for the association with *C. spectabilis* and corn DKB 290, with 88% and 78%, respectively, followed by *C. ochroleuca* and millet ADR300, with 62%, *U. ruziziensis*, 57%, and finally millet ADRG9050, with 23% reduction in the reproduction of *P. brachyurus*. Similar results were observed for the number of nematodes per gram of root, whose reproduction reductions in the soybean crop ranged from 17% for millet ADRG9050 to 80% for corn and *C. spectabilis* (Table 5).

These results corroborate the information that Oliveira et al. (2019) found on the effect of *B. subtilis* on *P. brachyurus*. In the study, the authors obtained an almost 88% reduction in nematode reproduction factor 60 days after sowing soybean seeds treated with *B. subtilis*.

Table 5. *Pratylenchus brachyurus* total in soybean root after cultivation of cover crops, whose seeds were treated or not treated with *Bacillus subitilis* and percentage of nematode reduction within each cover crop, at 70 days after sowing.

Treatments	Total nematode		
	Without *B. subtilis*	With *B. subtilis*	% Reduction
Corn	71817 aA	15712 bB	78
Millet ADRG 9050	14656 dA	11279 cB	23
Millet ADR 300	17388 cA	6543 dB	62
U. ruziziensis	41090 bA	17517 aB	57
C. ochroleuca	5790 eA	2194 eB	62
C. spectabilis	3263 fA	406 fB	88
	Nematode per gram of root		
Corn	26198 aA	5295 bB	80
Millet ADRG 9050	6087 dA	5077 bB	17
Millet ADR 300	7541 cA	2176 cB	71
U. ruziziensis	20495 dA	5809 aB	72
C. ochroleuca	1315 eA	1064 dB	19
C. spectabilis	985 fA	201 eB	80
p-value	$<2.2 \times 10^{-16}$		

Means followed by the same lowercase letter in the column and uppercase letter in the line do not differ by the Tukey test at 5% probability. p = probability of error.

Costa, Pasqualli and Prevedello (2014) found that the *C. spectabilis*, even not treated with biocontrol agents, contributes significantly to reducing the *P. brachyurus* population. In addition to this contribution, the results show that *C. spectabilis*, when treated with *B. subtilis*, reduced the total nematode population and the population per gram of root by 88% and 80%, respectively.

Other results, such as those of Silva (2016), showed that Bacillus species had good effects when used together with cover crops, especially those that do not show resistance to all nematode species. An excellent example is the brachiaria, which efficiently manages root-knot and cyst nematodes but hosts *P. brachyurus*. In this case, the use of *U. ruziziensis* has been recommended because, among the brachiaria species, this is one of the least favorable to the multiplication of this nematode.

There was no statistical difference for the root fresh mass of plants treated with *B. subtilis*. At the same time, for the untreated, higher averages were observed for treatments with both *Crotalaria* species (Table 6).

Table 6. Root fresh mass of soybean parasitized by *Pratylenchus brachyurus*, cultivated after cover crops, with or without treatment with *Bacillus subtilis*.

Treatments	Root fresh mass (g) - With *B. subtilis*	Root fresh mass (g) - Without *B. subtilis*
Corn DKB 290	3.29 aA	2.51 bA
Millet ADRG 9050	2.25 aA	2.49 bA
Millet ADR 300	2.86 aA	2.58 bA
U. ruziziensis	2.66 aA	1.98 bA
C. ochroleuca	2.92 aB	4.09 aA
C. spectabilis	3.23 aA	3.35 aA
CV (%)	36.13	

Means followed by the same letter in the column do not differ by the Tukey test at 5% probability. CV= coefficient of variation.

In the experiment with *B. subtilis* and *M. incognita*, it was possible to notice that in the presence of the bacterium, all the cover crops reduced the multiplication of the nematode, with control for the total number of nematodes varying from 24.5% to 96.3% and the number of nematodes per gram of root ranging from 24.9% to 96.3% (Table 7). On the other hand, in the absence of treatment with *B. subtilis*, there was an increase in the multiplication of the nematode in soybean cultivated after cover crops concerning corn.

Table 7. *Meloidogyne incognita* total and per gram of soybean root after cultivation of cover crops, whose seeds were treated or not treated with *Bacillus subitilis* and percentage of nematode reduction within each cover crop, at 60 days after sowing.

Treatments	With *B. subtilis*	Without *B. subtilis*	With *B. subtilis*	Without *B. subtilis*	% Reduction by *B. subtilis*
	Total nematode		% Reduction of coverage concerning the control		
Corn	50964 aB	755 dA	-	-	-
Millet ADRG 9050	1896 eA	1944 bA	96.3	-	2.5
Millet ADR 300	38326 bB	10912 aA	24.5	-	-
U. ruziziensis	15330 cB	1357 cA	69.9	-	-
C. spectabilis	3098 dB	666 eA	93.9	-	-
p-value	$2.2e^{-16}$				
Treatments	Nematode/g of root		% Reduction of coverage concerning the control		% Reduction by *B. subtilis*
Corn	6149 aB	63 dA	-	-	-
Millet ADRG 9050	253 eA	268 bA	95.9	-	5.6
Millet ADR 300	4616 bB	1198 aA	24.9	-	-
U. ruziziensis	1764 cB	190 cA	71.3	-	-
C. spectabilis	281 dB	50 eA	95.4	-	-
p-value	$2.2e^{-16}$				

Means followed by the same lowercase letter in the column and uppercase letter in the line do not differ by the Tukey test at 5% probability. p = probability of error.

Interestingly, for soybean grown after corn treated with *B. subtilis*, nematode reproduction was very pronounced (Table 7), and here, it is hypothesized that the association with the bacterium may have altered corn susceptibility to the nematode. Once again, the possibility of an endophytic activity of this bacterium with the studied plants must be addressed. Despite limited research, it is known that endophytic organisms establish a very restricted interaction with their host. To colonize the cortex cells, they need to silence the defense genes of plants temporarily. During this period, the plant may be fully exposed to the attack of pathogens, and here, as the initial inoculum was high (4000 eggs + J2), there may have been a massive infection of the roots. Although the information on the treatment of cover crops seeds was not elucidated, Basyony and Abo-Zaid (2018) observed that the application of *B. subtilis* could reduce the number of galls and the egg mass of *M. incognita*.

CONCLUSION

The association of *B. subitilis* and *B. methylotrophicus* with *C. spectabilis*, *U. ruziziensis,* and Millet ADR 300 confers an additional effect on *P. brachyurus* control. A pronounced effect on this reduction is observed in the association of *B. subtilis* with the grasses used in this study.

For the nematode *M. incognita*, the association was successful only for combining *C. spectabilis* with *B. methylotrophicus*. Although DKB 290 corn is not a cover crop, it is noted that when previously treated with *B. methylotrophicus*, the nematode population per gram of root is reduced by 90% in soybeans grown in succession.

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