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Research Article

Biological control of phytoparasitic nematodes in sugarcane fields[1]

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ABSTRACT

The management of nematode infested areas includes several measures, among which chemical nematicides are the most used. However, since their use is associated with environmental impacts, researches with biocontrol agents have acquired a greater importance. This study aimed to evaluate the performance of *Bacillus subtilis* + *B. licheniformis*, when applied to the planting furrow, on nematode control and its effects on sugarcane yield. Five experiments were carried out in infested areas, with four treatments: control - no nematicide; carbosulfan 700EC 4 L ha^{-1} - standard treatment; *B. subtilis* + *B. licheniformis* at 0.16 kg ha^{-1} and 0.20 kg ha^{-1}. Carbosulfan was more efficient in controlling the nematodes, reducing the populations at least four months after planting. The treatments with *B. subtilis* + *B. licheniformis* were more effective in the control of *Meloidogyne javanica*, when compared to *Pratylenchus*. The plots treated with carbosulfan produced 11 % more than the control. There was no difference between the *B. subtilis* + *B. licheniformis* doses, in relation to yield. The plots treated with the biological product produced 5 % more than the control.

KEYWORDS: *Bacillus subtilis*, *Bacillus licheniformis*, *Meloidogyne*, *Pratylenchus*.

RESUMO

Controle biológico de nematoides fitoparasitos em canaviais

O manejo de áreas infestadas por nematoides inclui uma série de medidas, dentre as quais os nematicidas químicos representam a mais utilizada. No entanto, visto que o uso desses produtos está associado a impactos ao ambiente, pesquisas com produtos biológicos têm adquirido grande importância. Objetivou-se avaliar o desempenho de *Bacillus subtilis* + *B. licheniformis*, quando aplicado ao sulco de plantio, no controle de nematoides e os efeitos na produtividade de canaviais. Foram conduzidos cinco experimentos em áreas infestadas, com quatro tratamentos: testemunha - sem nematicida; carbosulfan 700EC 4 L ha^{-1} - tratamento padrão; *B. subtilis* + *B. licheniformis* a 0,16 kg ha^{-1} e 0,20 kg ha^{-1}. O carbosulfan foi mais eficiente no controle dos nematoides, reduzindo as populações pelo menos até quatro meses após o plantio. Os tratamentos com *B. subtilis* + *B. licheniformis* foram mais efetivos no controle de *Meloidogyne javanica* do que de *Pratylenchus*. As parcelas tratadas com carbosulfan produziram 11 % mais que a testemunha. Não houve diferenças entre as doses de *B. subtilis* + *B. licheniformis*, em relação à produtividade. Parcelas tratadas com o produto biológico produziram 5 % mais que a testemunha.

PALAVRAS-CHAVES: *Bacillus subtilis*, *Bacillus licheniformis*, *Meloidogyne*, *Pratylenchus*.

INTRODUCTION

Sugarcane is parasitized by many phytoparasitic species in Brazil, although *Pratylenchus zeae* Graham, *P. brachyurus* (Godfrey) Filipjev & Schuurmans Stekhoven, *Meloidogyne javanica* (Treub) Chitwood and *M. incognita* (Kofoid & White) are the most important ones, due to their damage to the crop, which varies as a function of the occurring species, population, soil type, cultivar and many other factors (Dinardo-Miranda 2018). On average, nematodes cause yield losses of approximately 20-40 % in the first cycle (plant-cane), reaching more than 50 % in cases of high population and very susceptible cultivars (Dinardo-Miranda & Ferraz 1991, Dinardo-Miranda et al. 1996, Dinardo-Miranda et al. 1998, Dinardo-Miranda et al. 2008, Regis & Moura 1989). For ratoon, the yield is also reduced, compromising the crop longevity (Dinardo-Miranda & Menegatti 2004, Dinardo-Miranda et al. 2008, Dinardo-Miranda et al. 2010, Silva et al. 2006).

The management of infested sugarcane areas is carried out with several control tools, among which nematicides are undoubtedly the most used method,

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both for planting and ratoon (Dinardo-Miranda 2018).

The most used nematicides in sugarcane are the chemical ones, which rapidly reduce nematode populations and protect the growing plant from damage. Although these products maintain a low population of nematodes for only a few months after application, they contribute to significant increases in yield in infested areas, when compared to untreated areas (Dinardo-Miranda & Mengatti 2004, Dinardo-Miranda et al. 2008, Dinardo-Miranda et al. 2010, Silva et al. 2006). However, since chemical nematicides in the soil are associated with environmental impacts, researches with biocontrol agents have acquired a greater importance. In this context, rhizosphere microorganisms known as rhizobacteria have been able to promote substantial protection against nematodes parasitism (Siddiqui et al. 2001), especially *Bacillus subtilis* (Almagharabi et al. 2013, Rao et al. 2017, Soliman et al. 2019).

Cardoso & Araújo (2011) applied *B. subtilis* to the soil and observed an increase in sugarcane growth and a decrease in the reproduction of *Meloidogyne* sp. in the plants roots. The application of *B. subtilis* to the soil also reduced the reproduction of *Meloidogyne* spp. and *P. zeae* in the soil, and, in the sugarcane roots, the nematodes population in the plots treated with the biological product was similar to the population in plots with chemical treatment (carbofuran) (Morgado et al. 2015). Both experiments were carried out under greenhouse conditions.

The good performance of *B. subtilis* was also observed in a sugarcane naturally infested field, where Mazzuchelli et al. (2020) observed that the application of *B. subtilis* to the furrow provided the effective control of *Meloidogyne* sp. and *Pratylenchus* spp., providing an increase in yield in the treated plots.

The effect of *B. licheniformis* on nematode populations has also been reported. Calagiero at al. (2018) conducted a greenhouse assay in which the bacteria were introduced to the soil on tomato seedlings, at five hours before the inoculation with *M. incognita* juveniles, and observed that *B. licheniformis* significantly reduced the nematode densities in the roots. Du et al. (2022) also conducted a greenhouse assay in which tomato seedlings were planted in pots infested by *M. incognita*, confirming that *B. licheniformis* controlled the root-knot nematode and reduced its negative effect on tomato growth.

Despite the good results for sugarcane, part of the cited studies was conducted under greenhouse conditions or involved only *B. subtilis*. There are no results in the literature for the mix of *B. subtilis* + *B. licheniformis*. Therefore, this study aimed to evaluate the effect of *Bacillus subtilis* + *B. licheniformis* on nematode control and sugarcane yield, when applied to the planting furrow.

MATERIAL AND METHODS

Five experiments were conducted in areas with sandy soil, naturally infested by nematodes, in the São Paulo state, Brazil. The location where each experiment was conducted, as well as the planting date and cultivar used, are shown in Table 1. All experiments were arranged in a randomized blocks design, with five (experiments 1 and 5) or six (experiments 2, 3 and 4) replicates. The plots were represented by 6 furrows with 12 m, spaced apart at 1.5 m.

The following treatments were evaluated: control (no nematicide); carbosulfan (Marshal Star 700EC®) at 4 L ha^{-1} (standard treatment); *Bacillus subtilis* + *B. licheniformis* (Quartzo®) at 0.16 kg ha^{-1} and at 0.20 kg ha^{-1}. All nematicide treatments were applied in the planting furrow with a CO_2 pressurized backpack sprayer equipped with a 11003 spray tip, at a working pressure of 30 PSI, for a flow of 150 L ha^{-1}. Immediately after the nematicide application, the furrows were covered with soil.

Table 1. Location, planting date and cultivar of the experiments.

Experiment	Location (São Paulo state, Brazil)	Plantig date	Cultivar
1	Santa Cruz das Palmeiras	07 December 2016	CTC11
2	Serrana	10 January 2017	CTC4
3	Olímpia	21 February 2017	RB867515
4	Araras	10 March 2017	SP80-1816
5	Lençóis Paulista	30 May 2017	RB975357

The nematode populations were evaluated at 2, 4 and 10 months after planting. For this, plant roots and soil were collected from the first and sixth furrow in each plot and sampling, and the nematodes were extracted by a combination of sieving and centrifugation with sucrose solution (Jenkins 1964, Coolen & D'Herde 1972).

The yield of each plot was obtained at approximately 14 months after planting, considering stalks from the second to the fifth furrows. In this case, the yield was obtained by the biometric method (Landell et al. 1999).

For statistical analysis, the population data were transformed by the square root of (x + 1). All data were subjected to analysis of variance and means compared by the t test at 10 % of significance, using the SAS software (SAS Institute 2000).

After the analysis of each experiment, the data were jointly analyzed. Since the number of replications was not the same for all experiments, for these analyses, each experiment was considered as one replication, using the mean data for each parameter and treatment (Gomes 1982).

RESULTS AND DISCUSSION

Among the most important nematode species for sugarcane, *Pratylenchus zeae* was observed in all experimental fields, while *P. brachyurus* was observed in the experiment 2 and *Meloidogyne javanica* in the experiments 3 and 5.

There were no differences among the treatments, in relation to nematode (*P. zeae*) population, only in the experiment 1, both in the roots and in the soil (Tables 2 to 6). Despite that, the yield was higher in plots treated with carbosulfan, which differed significantly from the control (Table 2). This yield increase in plots treated with carbosulfan may be the result of a reduction in nematode population, in the two months prior to the first sampling. Novaretti et al. (1984) and Dinardo-Miranda (2018) reported that the warmer and rainier the planting period, the shorter the period in which chemical nematicides remain effective, as they can be leached and metabolized by plants more quickly, when compared to planting conducted in drier periods. As the experiment 1 was planted in December, it was subjected to a greater rainfall volume in the first two months after planting than the other experiments (Table 7), what justifies that, at two months after planting, when the first sampling was carried out, there was not a significant reduction in nematode populations in the plots treated with carbosulfan. Since the yield was higher in plots treated with this nematicide, when compared to the control, it is assumed that carbosulfan reduced the nematode populations until two months after planting, when the first sampling was carried out.

Table 2. Adult and juvenile population of *Pratylenchus zeae* in the roots (50 g) and in the soil (1 L) at 2, 4 and 10 months after planting, and stalks yield at the harvest (SYH, t ha^{-1}), according to the nematicide treatments. Experiment 1.

Treatment	2 months		4 months		10 months		SYH
	Roots	Soil	Roots	Soil	Roots	Soil	
Control	3,152 a	300 a	9,310 a	720 a	7,365 a	432 a	103 b
Carbosulfan (4 L ha^{-1})	2,656 a	252 a	11,310 a	768 a	13,146 a	420 a	115 a
Bs + *Bl** (0.16 kg ha^{-1})	1,844 a	444 a	11,090 a	816 a	18,163 a	264 a	105 b
Bs + *Bl** (0.2 kg ha^{-1})	3,328 a	504 a	12,232 a	936 a	10,481 a	276 a	112 ab

* *Bacillus subtilis* + *B. licheniformis*. Means within the same column followed by the same letter are not significantly different (t test; p ≤ 1).

Table 3. Adult and juvenile population of *Pratylenchus zeae* + *P. brachyurus* in the roots (50 g) and in the soil (1 L) at 2, 4 and 10 months after planting, according to the nematicide treatments. Experiment 2.

Treatment	2 months		4 months		10 months	
	Roots	Soil	Roots	Soil	Roots	Soil
Control	7,988 a	870 bc	10,729 a	660 a	4,450 a	960 a
Carbosulfan (4 L ha^{-1})	5,094 b	1,150 ab	6,258 b	600 a	4,317 a	780 a
Bs + *Bl** (0.16 kg ha^{-1})	6,258 b	670 c	9,957 ab	670 a	6,292 a	560 a
Bs + *Bl** (0.2 kg ha^{-1})	6,424 ab	1,320 ab	9,100 ab	780 a	4,083 a	590 a

* *Bacillus subtilis* + *B. licheniformis*. Means within the same column followed by the same letter are not significantly different (t test; p ≤ 1).

In the experiments 2 to 4, the treatment with carbosulfan reduced the population of *P. zeae* or *P. zeae* + *P. brachyurus* in the roots at least until four months after planting, while, in the experiment 5, the treatment with carbosulfan reduced the population of *M. javanica* in the roots until four months after planting (Tables 3 to 6). The treatments with *B. subtilis* + *B. licheniformis* were less effective than carbosulfan to reduce the population of *P. zeae* or *P. zeae* + *P. brachyurus*, although a smaller population of *P. zeae* + *P. brachyurus*, if compared to the control, was observed in the roots at two months after planting in the experiment 2 (Table 3). Likewise, in several occasions, such as in the second sampling in the experiment 3 (Table 4) and experiment 5 (Table 6), the population of *M. javanica* in the roots of plants treated with the biological product was lower than that observed in the control. These data suggest that the mixture of *B. subtilis* + *B. licheniformis* reduced the nematode population.

Yield was not obtained from the experiment 2. In the experiment 3, although the population of *P. zeae* decreased in the plots treated with carbosulfan at least until four months after planting, there were no differences between the treatments regarding yield. This occurred because RB867515, the variety planted in the experiment 3, is tolerant to *P. zeae* (Dinardo-Miranda et al. 2019). In the experiments 4 and 5, in which the smallest nematode populations at the beginning of the development of the crop

Table 4. Second-stage juvenile population *of Meloidogyne javanica* (Mj) and adult and juvenile population of *Pratylenchus zeae* (Pz) in the roots (50 g) and in the soil (1 L) at 2, 4 and 10 months after planting, and stalks yield at the harvest (SYH, t ha^{-1}), according to the nematicide treatments. Experiment 3.

Treatment	2 months				4 months				10 months				SYH
	Roots		Soil		Roots		Soil		Roots		Soil		
	Mj	Pz	Mj	Pz	Mj	Pz	Mj	Pz	Mj	Pz	Mj	Pz	
Control	85 a	6,189 a	10 a	920 b	8,873 a	25,770 a	210 a	690 ab	217 a	2,518 a	30 a	470 a	132 a
Carbosulfan (4 L ha^{-1})	33 a	1,433 b	0 a	1,040 b	350 b	8,526 b	240 a	410 b	100 a	2,325 a	0 a	1,150 a	133 a
Bs + *Bl** (0.16 kg ha^{-1})	58 a	5,275 a	0 a	2,760 a	203 b	11,110 ab	250 a	980 a	0 a	2,917 a	50 a	600 a	133 a
Bs + *Bl** (0.2 kg ha^{-1})	0 a	8,827 a	0 a	2,270 a	4,091 ab	11,259 ab	50 a	690 ab	17 a	1,750 a	0 a	460 a	139 a

* *Bacillus subtilis* + *B. licheniformis*. Means within the same column followed by the same letter are not significantly different (t test; p ≤ 1).

Table 5. Adult and juvenile population of *Pratylenchus zeae* in the roots (50 g) and in the soil (1 L) at 2, 4 and 10 months after planting, and stalks yield at the harvest (SYH, t ha^{-1}), according to the nematicide treatments. Experiment 4.

Treatment	2 months		4 months		10 months		SYH
	Roots	Soil	Roots	Soil	Roots	Soil	
Control	4,124 a	180 a	1,358 a	180 a	933 b	50 b	103 c
Carbosulfan (4 L ha^{-1})	416 b	120 a	233 b	90 b	1,650 ab	110 ab	121 a
Bs + *Bl** (0.16 kg ha^{-1})	3,770 a	200 a	825 a	110 ab	1,892 a	200 a	113 ab
Bs + *Bl** (0.2 kg ha^{-1})	2,731 a	80 a	1,125 a	140 ab	1,633 ab	160 ab	106 bc

* *Bacillus subtilis* + *B. licheniformis*. Means within the same column followed by the same letter are not significantly different (t test; p ≤ 1).

Table 6. Second-stage juvenile population *of Meloidogyne javanica* (Mj) and adult and juvenile population of *Pratylenchus zeae* (Pz) in the roots (50 g) and in the soil (1 L) at 2, 4 and 10 months after planting, and stalks yield at the harvest (SYH, t ha^{-1}), according to the nematicide treatments. Experiment 5.

Treatment	2 months				4 months				10 months				SYH
	Roots		Soil		Roots		Soil		Roots		Soil		
	Mj	Pz	Mj	Pz	Mj	Pz	Mj	Pz	Mj	Pz	Mj	Pz	
Control	22 ab	70 a	0 a	36 a	5,940 a	2,180 a	852 a	144 a	230 ab	1,410 a	360 a	516 a	65 b
Carbosulfan (4 L ha^{-1})	30 ab	43 a	0 a	0 a	550 b	2,630 a	0 b	36 a	120 b	520 b	48 a	228 a	77 a
Bs + *Bl** (0.16 kg ha^{-1})	0 b	46 a	36 a	0 a	20 b	3,710 a	48 b	72 a	4,970 a	1,110 ab	120 a	384 a	71 ab
Bs + *Bl** (0.2 kg ha^{-1})	43 a	62 a	0 a	12 a	2,710 ab	2,870 a	324 ab	96 a	2,130 a	1,620 ab	48 a	336 a	70 ab

* *Bacillus subtilis* + *B. licheniformis*. Means within the same column followed by the same letter are not significantly different (t test; p ≤ 1).

were observed in plots treated with carbosulfan, the highest yields were also observed for this treatment, differing significantly from the control. In these two experiments, the yield in treatments with *B. subtilis* + *B. licheniformis* did not differ from the control or the treatment with carbosulfan.

The performance of the treatments can be better evaluated by analyzing the nematode population data from five experiments and yield data from four experiments (Table 8). Carbosulfan was the most efficient treatment for nematode control, reducing the populations of *P. zeae* or *P. zeae* + *P. brachyurus* in the plant roots at least until four months after planting. Since the population of *M. javanica* was low at the first sampling (two months after planting), the efficiency of carbosulfan could be noticed in the second sampling, carried out at four months after planting, when the population of *M. javanica* in the plant roots treated with carbosulfan was smaller, when compared to the roots from the control treatment (Table 8).

The *B. subtilis* + *B. licheniformis* treatments were less effective in the control of *M. javanica* and *Pratylenchus* than carbosulfan, at least for the first two months after planting. At four months after planting, the populations of *Pratylenchus* in the plant roots treated with the biological nematicide did not differ from the plants treated with carbosulfan, although they also did not differ from the control treatment. However, populations of *M. javanica* in the roots of plants treated with *B. subtilis* + *B. licheniformis* were smaller than in plants from the control plots, being similar to the population observed in the plant roots treated with carbosulfan. These data suggest that the *B. subtilis* + *B. licheniformis* treatments were more effective on *M. javanica* than on *Pratylenchus*.

According to researchers, bacteria belonging to the *Bacillus* genus may present different action mechanisms on phytoparasitic nematodes, such as the production of toxic metabolites, interference in host recognition, competition for nutrients and induction of resistance in plants (Lian et al. 2007, Oliveira et al. 2014, Yu et al. 2015, Zheng et al. 2016). All these mechanisms contribute to reduce the number of nematodes inside the roots, as observed in the present study.

Due to the reduction in the nematode population, the plots treated with carbosulfan or *B. subtilis* + *B. licheniformis* produced significantly more than the control plots. Since carbosulfan was more efficient in nematode control, at least until four months after planting, the highest yield was observed in this treatment, followed by treatments with *B. subtilis* + *B. licheniformis.* Since young plants are more severely damaged by nematodes than older ones (Kayani et al. 2018), carbosulfan promoted a greater reduction in nematode populations in the first two months of planting, when compared to treatments with *B. subtilis* + *B. licheniformis*, explaining the higher yield in the plots treated with carbosulfan than in those treated with the biological nematicide. The plots treated with carbosulfan produced on average 11 % more than the control plots. There was no difference between the *B. subtilis* + *B. licheniformis* doses, in relation to yield, and the plots treated with the biological product produced 5 % more than the control (Table 8).

Table 7. Total rainfall (mm) in each experiment, according to the crop age.

Experiment	1 month	2 months	3 months	4 months
1	156.1	347.2	452.4	593.8
2	154.6	251.9	336.7	400.7
3	80.1	146.8	213.4	340.0
4	117.7	209.0	336.1	360.4
5	0	0	0	22.4

Table 8. Second-stage juvenile population *of Meloidogyne javanica* (Mj) and of adult and juvenile *Pratylenchus* (Pspp) in the roots (50 g) and in the soil (1 L) at 2, 4 and 10 months after planting, and stalks yield at the harvest (SYH, t ha^{-1}), according to the nematicide treatments. Mean of five experiments.

Treatment	2 months				4 months				10 months				SYH
	Roots		Soil		Roots		Soil		Roots		Soil		
	Mj	Pspp	Mj	Pspp	Mj	Pspp	Mj	Pspp	Mj	Pspp	Mj	Pspp	
Control	22 a	4,324 a	3 b	461 a	2,966 a	9,869 a	212 a	479 a	88 a	3,335 b	78 a	486 a	102 c
Carbosulfan (4 L ha^{-1})	13 a	1,928 b	39 a	512 a	184 b	5,805 b	50 a	379 b	43 a	4,392 ab	11 a	537 a	118 a
Bs + *Bl** (0.16 kg ha^{-1})	13 a	3,438 a	0 b	814 a	47 b	7,838 ab	60 a	530 a	1,017 a	6,074 a	24 a	402 a	107 b
Bs + *Bl** (0.2 kg ha^{-1})	29 a	4,294 a	11 ab	837 a	1,362 b	7,317 ab	75 a	529 a	432 a	3,913 ab	34 a	364 a	113 ab

* *Bacillus subtilis* + *B. licheniformis*. Means within the same column followed by the same letter are not significantly different (t test; p ≤ 1).

The results of these experiments partially corroborate those by Cardoso & Araújo (2011) and Morgado et al. (2015), who had already reported that the application of *B. subtilis* to the soil reduces the reproduction factor of *Meloidogyne* spp. and *P. zeae* in sugarcane. However, these authors conducted their studies in a greenhouse, not in an infested field.

The results of the present study also partially corroborate those obtained by Mazzuchelli et al. (2020). Although the authors worked with *B. subtilis* (AP-3 strain, 10^9 cel ha^{-1}), they reported that the biological product was more efficient than the chemical nematicide (carbofuran 8 L ha^{-1}) on the control of *Meloidogyne* and *Pratylenchus*, reducing the nematodes population during the ratoon cycle. Despite that, they did not observe yield increases due to the reduction of the nematodes population. In the present study, the chemical nematicide was more efficient than the biological one, and, in both cases, the nematode population was high in all plots at ten months after planting, including in the control. Moreover, in the present study, the nematode population reduction was followed by an increased yield.

CONCLUSIONS

1. Carbosulfan was more efficient than *Bacillus subtilis* + *B. licheniformis* to reduce populations of *Pratylenchus*, at least until four months after planting;
2. The treatments with *B. subtilis* + *B. licheniformis* were more effective for the control of *Meloidogyne javanica*, when compared to *Pratylenchus*;
3. Carbosulfan and *B. subtilis* + *B. licheniformis* promoted increases of 11 and 5 % in yield, respectively.

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