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Crop rotation combined with bionematicides for *Pratylenchus brachyurus* management in soybean

Rotação de culturas combinada com bionematicidas para o manejo de *Pratylenchus brachyurus* na soja

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Highlights

Cover crops are efficient in controlling *P. brachyurus* in successive soybean crops.
Bionematicides based on fungi and bacteria contribute to *P. brachyurus* control.
Cultural and biological methods have additive effects on *P. brachyurus* populations.
Integrated management can improve soybean grain yield.

Abstract

The integration of nematode management strategies is an increasingly relevant focus of research. This study assessed the efficiency of cover crops combined with bionematicides in managing *Pratylenchus brachyurus* in soybean. The experiment used pots with a soil-sand mix. Soybean cultivar M6410 IPRO was planted and inoculated with 500 *P. brachyurus* per pot. At the end of the soybean cycle (R8 stage), they were harvested and the pots sown with the P3858 PWU maize hybrid, *Urochloa ruziziensis* (Ur), Ur + millet, Ur + pigeon pea, Ur + *Macrotyloma axillare* 'Java', Ur + buckwheat, or a seed mix containing Ur, millet, pigeon pea, and buckwheat. After 85 days, the cover crops were cut, and wheat was grown for 110 days. After wheat harvest, pots were sown with untreated soybean seeds (control) or seeds treated with *Bacillus subtilis* + *Bacillus licheniformis* + *Purpureocillium lilacinum,* or *P. lilacinum* + *Trichoderma harzianum.* The experiment was conducted in a completely randomized design, using a 7 (cover crop) × 3 (bionematicide) factorial scheme. After 110 days (2022 trial), soybean crops were harvested and evaluated for nematological variables and grain yield. The process was repeated for the 2023 trial. Cover crops and bionematicides significantly reduced *P. brachyurus,* with 73.8% control in roots and 76.4% in

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soil in 2022, and 76.4% in roots and 90.7% in soil in 2023. These practices also increased grain yields by 43.9 to 65.7% in the 2023 season.
Key words: Biological control. Cover crop. Crop system. Lesion nematode.

Resumo

A integração de estratégias de manejo de nematoides tornou-se foco de pesquisas cada vez mais relevantes. Este estudo avaliou a eficiência de plantas de cobertura combinadas com bionematicidas no manejo de *Pratylenchus brachyurus* em soja. O experimento foi conduzido em vasos contendo uma mistura de solo e areia. A cultivar de soja M6410 IPRO foi semeada e inoculada com 500 espécimes de *P. brachyurus* por vaso. Ao final do ciclo da soja, quando as plantas atingiram o estádio R8, foram colhidas, e os vasos foram semeados com milho P3858 PWU, *Urochloa ruziziensis* (Ur), Ur + milheto, Ur + guandu, Ur + *Macrotyloma axillare* 'Java', Ur + trigo-sarraceno, ou uma mistura de sementes contendo Ur, milheto, guandu e trigo-sarraceno. Após 85 dias, as plantas de cobertura foram cortadas, e o trigo foi cultivado por 110 dias. Após a colheita do trigo, os vasos foram semeados com sementes de soja sem tratamento (controle) ou tratadas com *Bacillus subtilis* + *Bacillus licheniformis* + *Purpureocillium lilacinum,* ou *P. lilacinum* + *Trichoderma harzianum.* O experimento seguiu um delineamento inteiramente casualizado, com arranjo fatorial 7 (plantas de cobertura) × 3 (bionematicidas). Após 110 dias (ensaio de 2022), as plantas de soja foram colhidas e avaliadas quanto a variáveis nematológicas e produtividade de grãos. O experimento foi repetido no ensaio de 2023. As plantas de cobertura e os bionematicidas reduziram significativamente as populações de *P. brachyurus,* com controle de 73,8% nas raízes e 76,4% no solo em 2022, e 76,4% nas raízes e 90,7% no solo em 2023. Essas práticas também aumentaram a produtividade de grãos entre 43,9% e 65,7% na safra de 2023.
Palavras-chave: Controle biológico. Cultura de cobertura. Sistema de cultivo. Nematoide das lesões.

Introduction

Pratylenchus brachyurus, the root lesion nematode, is one of the most important soybean parasites. Its wide range of hosts, including maize, forage grasses, and weeds, makes it challenging to design crop rotation strategies. For effective control of these parasites, it is recommended that an integrated management approach that combines nematode-reducing plants and biological methods be used (Dallemole-Giaretta et al., 2011; Araujo et al., 2023).

There is an ongoing search for plant species that can reduce nematode populations in the off-season. The rationale behind this strategy is to minimize damage to crops grown in successive seasons and obtain additional benefits, such as increased soil organic matter and improved physicochemical properties (Gabriel et al., 2018; Barbieri et al., 2019). Cover crops such as pearl millet (*Pennisetum glaucum*) (Timper & Hanna, 2005; Amorim et al., 2019), buckwheat (*Fagopyrum esculentum*) (Melo et al., 2023), pigeon pea (*Cajanus cajan*)

(Santana-Gomes et al., 2019), *Macrotyloma axillare* 'Java' (Miamoto et al., 2021), and *Urochloa ruziziensis* (Dias-Arieira et al., 2021) provide multiple benefits to cropping systems, including nematode control.

Cover crops create an environment conducive to the proliferation of biocontrol agents (Kabir & Koide, 2002; Dallemole-Giaretta et al., 2011; Singhal et al., 2020). Biological control is currently the most widely used method for nematode management in soybean. Biocontrol agents are typically fungi or bacteria, and fungal agents include saprophytic and opportunistic species that parasitize nematode eggs, such as *Purpureocillium lilacinum* (=*Paecilomyces lilacinus*), *Pochonia chlamydosporia,* and *Trichoderma* spp. (Manzanilla-López et al., 2013; Yang et al., 2015). On the other hand, bacterial agents, especially *Bacillus* spp., act by forming a metabolically active biofilm in the rhizoplane, resulting in altered rhizosphere characteristics and antibiosis (Hu et al., 2017; Hashem et al., 2019). It should be noted that, despite having different mechanisms of action, both fungal and bacterial agents can activate plant defenses and promote vegetative growth (Hu et al., 2017; Ghorbanpour et al., 2018; Hashem et al., 2019).

Combining off-season cover cropping with bionematicide treatment may be an effective solution to control nematodes in soybean (Amer-Zareen et al., 2004; Araujo et al., 2023), with the added benefit of improving yield gains. As such, this study aimed to assess the use of different cover crops combined with bionematicides for *P. brachyurus* management in soybean crops.

Materials and Methods

General experimental procedures

The experiment was conducted in an open area (23°47′06′′S 53°04′24′′W, 438 m a.s.l.) in Cruzeiro do Oeste, Parana state, Brazil, in two agricultural years. Each experimental unit consisted of a pot containing 5 kg of a 2:1 mixture of soil and sand. Previous analysis of 20 random samples of substrate confirmed the absence of nematodes. The soil was typical medium-textured dystroferric Red Latosol, containing 24% clay, 68% sand, and 8% silt (Empresa Brasileira de Pesquisa Agropecuária [EMBRAPA], 2006). The pots were placed on a pallet exposed to the elements, similar to natural cultivation conditions. In September 2020, one soybean M6410 IPRO seed was planted in each pot. Seeds were previously treated with Maxim XL (10 g L^{-1} metalaxyl-M + 25 g L^{-1} fludioxonil, Syngenta) at a rate of 100 mL 100 kg^{-1} seed and an inoculant based on *Bradyrhizobium elkanii* + *Bradyrhizobium japonicum* (5 × 10^9 CFU mL^{-1}) at 100 mL 50 kg^{-1} seed. At sowing, fertilization was performed according to recommendations for soybean crops, using 120 kg ha^{-1} P_2O_5 and 130 kg ha^{-1} K_2O. At 7 days after sowing (DAS), each plant was inoculated with 500 *P. brachyurus.* The nematode inoculum was obtained from a pure population maintained on soybean and extracted according to the method proposed by Hussey and Barker as adapted by Boneti and Ferraz (1981), but without sodium hypochlorite. The suspension was calibrated to 250 nematodes mL^{-1}. For inoculation, 2 mL of inoculum was deposited into two open holes in the soil near the base of the plant.

This procedure aimed to promote nematode reproduction in a manner that simulates natural infestation, with nematodes surviving in soil and root fragments. Soybean plants were grown to physiological maturity (110 days of cultivation).

The soybean shoots were cut and discarded. Each pot was then sown with one of the following treatments: maize (control), *Urochloa ruziziensis* (Ur), Ur + pigeon pea, Ur + buckwheat, Ur + pearl millet, Ur + Java, or a cover crop mix (Ur + pigeon pea + buckwheat + millet), with one plant of each species per pot. At 85 DAS, shoots were cut at a height of 20 cm from the ground and discarded, simulating grazing. Plants were cut again close to the ground, with the residues left on the soil. Next, the plant material was deposited in the respective pot, simulating the remaining ground cover after grazing. The pots were then sown with wheat cv. Quartzo seeds and fertilized with 80 kg ha^{-1} P_2O_5, 60 kg ha^{-1} K_2O, and 90 kg ha^{-1} N. Wheat crops were cultivated until the end of their cycle (approximately 110 days), this cultivar being selected for its FR value close to 1.0 (Gonçalves et al., 2018).

Pots were sown again with soybean M6410 IPRO and fertilized as described above. The following biological treatments were applied: Profix® (Agrivalle, *B. subtilis* + *B. licheniformis* + *P. lilacinum,* 200 g 100 kg^{-1} seed), Nemat® + Ecotrich® (Ballagro, *P. lilacinum* + *Trichoderma harzianum,* 250 g and 60 g 100 kg^{-1} seed) and an untreated control. The experiment was conducted in a completely randomized design, following a 7 × 3 factorial arrangement (seven rotation crops and three biological treatments).

At 110 DAS, when soybean plants were at the R8 stage, the entire root system and 100 cm³ of soil were collected for nematode analysis. The roots were washed and processed according to Hussey and Barker, as adapted by Boneti and Ferraz (1981) but without sodium hypochlorite. Nematodes were extracted from the soil samples by flotation extraction in sucrose solution (Jenkins, 1964). The resulting nematode suspensions were counted in a Peters chamber under an optical microscope. Results are expressed as number of *P. brachyurus* g^{-1} root or 100 cm^{-3} soil (population density).

At R8, plants were also evaluated for grain yield. Pods were harvested, placed in paper bags, and dried to constant weight in a forced-air oven at 65 °C. Grains were weighed on a semi-analytical scale. Grain yield, expressed as kg ha^{-1}, was calculated by multiplying the average grain weight per plant (kg) by the plant population per hectare (200,000 plants ha^{-1}). This experiment is hereafter referred to as the 2022 soybean trial.

After the soybean harvest, cover crop and wheat cycles were repeated. Soybean M6410 IPRO seeds were then treated with the aforementioned products and grown for 110 days until R8. For these analyses, the entire root system was collected, weighed, and submitted to the nematode extraction procedures. The number of nematodes was counted and divided by the root fresh weight to obtain the number of nematodes g^{-1} root. Soil samples (100 cm^3) were collected, nematodes extracted and quantified as previously described, and grain yield determined. This experiment is hereafter referred to as the 2023 soybean trial.

Statistical analysis

To meet the normality assumptions established by the Shapiro-Wilk test, the number of nematodes and nematodes per gram of root were square-root transformed. The data were submitted to analysis of variance at a 5% significance level. In the case of significant interactions, factors were analyzed together. When no interaction was identified, the main effects were evaluated, and means were compared by the Scott-Knott test at 5% significance. For analysis of population density data in the 2023 trial, significance was set at 7%. Statistical analyses were conducted using Sisvar software (Ferreira, 2014).

Results and Discussion

In the 2022 trial, there were significant interaction effects on *P. brachyurus* population density in soybean roots (Table 1). The combination of Ur + pigeon pea with *P. lilacinum* + *T. harzianum* or *B. subtilis* + *B. licheniformis* + *P. lilacinum* reduced population density by 79.1 and 87.4%, respectively. The cover crop mix combined with *B. subtilis* + *B. licheniformis* + *P. lilacinum* decreased nematode population density by 55.2%, compared with the untreated control.

In analyzing the effects of biological crop treatments (Table 1), *B. subtilis* + *B. licheniformis* + *P. lilacinum* combined with Ur + pigeon pea, Ur + Java, and the cover crop mix reduced nematode populations by 73.8, 38.0, and 55.2%, respectively, in relation to the control. In the untreated control, Ur, Ur + Java, and Ur + buckwheat differed from the other crops, with nematode reductions of 25.1, 10.2, and 58.3%, respectively, compared with maize.

Similar to that observed for soybean root population density, soil population density was influenced by the interaction effects of factors (Table 2). Ur + pigeon pea and Ur + buckwheat differed from the other crops in treatments with *P. lilacinum* + *T. harzianum,* with declines of 72.2 and 60.0%, respectively, compared with maize. Combining Ur, Ur + pigeon pea, Ur + Java and the cover crop mix with *B. subtilis* + *B. licheniformis* + *P. lilacinum* led to reductions of 57.1, 51.0, 68.4, and 46.1%, respectively, in relation to maize. *P. lilacinum* + *T. harzianum* combined with Ur, Ur + millet, and the cover crop mix provided decreases of 50.3, 76.4, and 46.2% compared with the control. On the other hand, *B. subtilis* + *B. licheniformis* + *P. lilacinum* combined with these cover crops led to reductions of 72.6, 64.4, and 65.4%, respectively, in relation to the control.

Table 1
Number of Pratylenchus brachyurus per gram of soybean root grown in succession to different cover crops and treated or not with biological nematicides in the 2022 and 2023 trials

Crop	*Purpureocillium lilacinum + Trichoderma harzianum*	*Bacillus subtilis + Bacillus licheniformis + P. lilacinum*	Untreated control
		2022	
Maize	320 aA	455 aA	489 aA
U. ruziziensis (Ur)	292 aA	343 aA	366 bA
Ur + millet	539 aA	500 aA	665 aA
Ur + pigeon pea	197 aB	119 bB	945 aA
Ur + Java	375 aA	282 bA	439 bA
Ur + buckwheat	376 aA	495 aA	204 bA
Mix[1]	838 aA	204 bB	714 aA
CV (%)		44.24	
		2023	
Maize	2945 aB	1799 aC	5086 aA
U. ruziziensis (Ur)	1051 bB	1733 aB	4447 aA
Ur + millet	1601 bA	1351 bA	2650 bA
Ur + pigeon pea	1078 bB	2527 aA	2403 bA
Ur + Java	2537 aA	923 bB	1932 bA
Ur + buckwheat	2072 aA	2300 aA	1856 bA
Mix[1]	3355 aA	1547 bB	2546 bA
CV (%)		25.14	

Means in columns followed by the same lowercase letter and means in rows followed by the same uppercase letter are not significantly different according to the Scott–Knott test ($p < 0.05$). Original means were transformed by $\sqrt{(x + 1)}$ prior to statistical analysis. [1]Mix: *U. ruziziensis* + buckwheat + pigeon pea +millet.

Table 2
Number of *Pratylenchus brachyurus* per 100 cm^{-3} of soil under soybean grown in succession to different cover crops and treated or not with biological nematicides in the 2022 and 2023 trials. Analyses were conducted110 days after soybean sowing

Crop	*Purpureocillium lilacinum + Trichoderma harzianum*	*Bacillus subtilis + Bacillus licheniformis + P. lilacinum*	Untreated control
		2022	
Maize	248 aA	282 aA	255 cA
U. ruziziensis (Ur)	219 aB	121 bB	441 bA
Ur + millet	167 aB	252 aB	708 aA
Ur + pigeon pea	69 bB	138 bA	196 cA
Ur + Java	240 aA	89 bA	168 cA
Ur + buckwheat	99 bB	296 aA	317 cA
Mix[1]	236 aB	152 bB	439 bA
CV (%)		29.83	
		2023	
Maize	553 aB	218 aB	1436 aA
U. ruziziensis (Ur)	222 bB	219 aB	2087 aA
Ur + millet	56 bB	82 aB	601 bA
Ur + pigeon pea	130 bB	424 aA	680 bA
Ur + Java	143 bB	78 aB	520 bA
Ur + buckwheat	307 aA	251 aA	420 bA
Mix[1]	433 aB	200 aB	807 bA
CV (%)		53.37	

Means in columns followed by the same lowercase letter and means in rows followed by the same uppercase letter are not significantly different according to the Scott–Knott test ($p < 0.05$). Original means were transformed by $\sqrt{(x + 1)}$ prior to statistical analysis. [1]Mix: *U. ruziziensis* + buckwheat + pigeon pea +millet.

In the 2023 trial, *P. brachyurus* soybean root population density (Table 1) declined by 63.4, 45.6, and 64.3% with the combination of Ur + pigeon pea, Ur + millet, and Ur with *P. lilacinum* + *T. harzianum,* respectively, compared with maize. *B. subtilis* + *B. licheniformis* + *P. lilacinum* combined with Ur + millet, Ur + Java and the cover crop mix prompted reductions of 24.9, 48.7, and 14.0%, respectively. In units without biological control, these cover crop systems reduced population density by 47.9 to 63.5% compared with maize, also differing significantly from Ur.

By analyzing the effect of biological cover crop treatments, it was possible to confirm the efficiency of bionematicides. In soybean planted after maize, *B. subtilis* + *B. licheniformis* + *P. lilacinum* and *P. lilacinum* + *T. harzianum* treatments decreased nematode population density by 64.6 and 42.1%, respectively (Table 1). Similarly, in soybean grown after Ur, these biological treatments caused reductions of 76.4 and 61.0%, respectively. In soybean planted after Ur + pigeon pea, *P. lilacinum* + *T. harzianum* reduced *P. brachyurus* population density by 55.1%. In soybean grown in succession to Ur + Java and cover crop mix, *B. subtilis* + *B. licheniformis* + *P. lilacinum* reduced nematode populations by 52.2 and 39.2%, respectively.

Bionematicides were also effective in reducing the soil population density of P. brachyurus (Table 2). In soybean grown in succession to Ur + Java, Ur + pigeon pea, Ur + millet, and Ur treated with *P. lilacinum* + *T. harzianum,* soil population density declined by 74.1, 76.5, 89.9, and 59.9%, respectively. On the other hand, cover crop treatment did not influence nematode populations in units treated with *B. subtilis* + *B. licheniformis* + *P. lilacinum.* Without biological treatments, cover crops decreased soil nematode populations by 47.9 to 62.5% in relation to maize, except for Ur.

The potential of some plants can be observed by analyzing the effect of cover crops in biological treatments. In the *B. subtilis* + *B. licheniformis* + *P. lilacinum* treatment, maize, Ur, Ur + millet, Ur + Java, and the cover crop mix reduced nematode root populations by 84.8, 89.5, 86.4, 85.0, and 75.2%, respectively, compared with the untreated control (Table 2). Similarly, P. lilacinum + T. harzianum decreased these populations by 61.5, 89.4, 90.7, 72.5, and 46.3%, respectively, in these crops. *P. lilacinum* + *T. harzianum* combined with Ur + pigeon pea provided 80.9% nematode control.

The factors did not exert significant interaction effects on soybean yield in the 2022 trial, but the main effects were significant. Soybean yield was higher when in succession to Ur when compared to the control, resulting in gains of 20.0% (Table 3).

Table 3

Yield and yield gain of soybean grown in succession to different cover crops or maize in the 2022 trial. Analyses were conducted 110 days after soybean sowing

Crop	Soybean yield (kg ha^{-1})	Yield gain (%)
Maize	1440 b	Reference
U. ruziziensis (Ur)	1728 a	20.0
Ur + millet	1398 b	-
Ur + pigeon pea	1332 b	-
Ur + Java	1446 b	-
Ur + buckwheat	1338 b	-
Mix1	1368 b	-
CV (%)	10.83	

Means in columns followed by the same lowercase letter are not significantly different according to the Scott–Knott test ($p < 0.05$). Original means were transformed by $\sqrt{x + 1}$ prior to statistical analysis. [1]Mix: U. ruziziensis + buckwheat + pigeon pea + millet.

Both biological treatments increased yields in relation to the control, by 17.2 and 14.9% for B. subtilis + B. licheniformis + P. lilacinum and P. lilacinum + T. harzianum, respectively (Table 4).

Table 4
Yield and yield gain of soybean grown in succession to different cover crops and treated or not with biological nematicides in the 2022 trial. Analyses were conducted 110 days after soybean sowing

Biological treatment	Soybean yield (kg ha^{-1})	Yield gain (%)
Untreated control	1290 b	Reference
Bacillus subtilis + B. licheniformis + Purpureocillium lilacinum	1512 a	17.2
P. lilacinum + Trichoderma harzianum	1482 a	14.9
CV (%)	10.83	

Means in columns followed by the same lowercase letter are not significantly different according to the Scott–Knott test ($p < 0.05$). Original means were transformed by √(x + 1) prior to statistical analysis.

There were significant interaction effects on soybean yield in the 2023 trial (Table 5). With the exception of Ur, all other cover crops combined with *P. lilacinum* + *T. harzianum* did not differ from maize with regard to soybean yield. When combined with *B. subtilis* + *B. licheniformis* + *P. lilacinum* treatment, cover crops did not differ from maize. In the absence of biological control, the highest yields were observed in soybean grown after maize or Ur. However, in analyzing the effect of bionematicides in cover crop systems, a 59.0, 43.9, and 65.7% yield gain was found with the combination of Ur + Java, Ur + buckwheat, and cover crop mix with *P. lilacinum* + *T. harzianum,* respectively, and 56.0, 51.7, and 59.1% for the respective crops combined with *B. subtilis* + *B. licheniformis* + *P. lilacinum.* Furthermore, the association between Ur + pigeon pea and *P. lilacinum* + *T. harzianum* prompted a 53.7 % yield gain.

Table 5
Yield and yield gain of soybean grown in succession to different cover crops and treated or not with biological nematicides in the 2023 trial. Analyses were conducted 110 days after soybean sowing. Yield gains were calculated in relation to the untreated control

Crop	*Purpureocillium lilacinum + Trichoderma harzianum*		*Bacillus subtilis + Bacillus licheniformis + P. lilacinum*		Untreated control
	Soybean yield (kg ha^{-1})	Yield gain (%)	Soybean yield (kg ha^{-1})	Yield gain (%)	Soybean yield (kg ha^{-1})
Maize	1800 aA	-	726 aA	-	1926 aA
U. ruziziensis (Ur)	1032 bB	-	1980 aA	14.2	1734 aA
Ur + millet	1548 aA	22.3	1296 aA	-	1266 bA
Ur + pigeon pea	2112 aA	53.7	1686 aB	22.7	1374 bB
Ur + Java	1746 aA	59.0	1722 aA	56.0	1098 bB
Ur + buckwheat	1770 aA	43.9	1866 aA	51.7	1230 bB
Mix[1]	1800 aA	65.7	1728 aA	59.1	1086 bB
CV (%)			14.18		

Means in columns followed by the same lowercase letter are not significantly different according to the Scott–Knott test ($p < 0.05$). Original means were transformed by √(x + 1) prior to statistical analysis. [1]Mix: *U. ruziziensis* + buckwheat + pigeon pea + millet.

The use of bionematicides combined with cover crops that are less susceptible to nematodes than maize can significantly reduce P. brachyurus populations in soybean succession. This association has shown good results (Araujo et al., 2023), especially when using plants that are not susceptible to nematodes. An example is *U. ruziziensis,* which is effective in managing *Meloidogyne javanica, Meloidogyne incognita, Heterodera glycines,* and *Rotylenchulus reniformis,* but susceptible to *P. brachyurus* (Carneiro et al., 2006; Dias-Arieira et al., 2021).

Urochloa ruziziensis is an interesting rotation crop, since this grass produces highly nutritional straw with the potential to persist in soil (Debiasi et al., 2016; Dias-Arieira et al., 2021). Its residues provide soil cover and increase the presence of roots in the system, facilitating root growth in succeeding crops and increasing soil organic matter (A. A. Balbinot Jr. et al., 2017; Dias-Arieira et al., 2021). Although this plant is susceptible to *P. brachyurus,* the nematode reproduction factor was lower than that of maize (Queiróz et al., 2014).

The findings of this study align with previous research demonstrating the effectiveness of *Urochloa ruziziensis* and millet in reducing *P. brachyurus* populations in succeeding soybean crops. Araujo et al. (2023) reported that *U. ruziziensis* combined with *Bacillus methylotrophicus* decreased nematode density by 53–67%, while millet ADR300 rotation using the same biological treatment resulted in a 69% decrease.

Similarly, the present study found that *U. ruziziensis* combined with *B. subtilis* + *B. licheniformis* + *P. lilacinum* reduced nematode populations in soybean roots by up to 89.5%, while *U. ruziziensis* + millet decreased them by 86.4%. Furthermore, both studies highlight the role of *B. subtilis* as an effective biocontrol agent, with Araujo et al. (2023) reporting reductions of up to 80% when applied with maize, millet, or *U. ruziziensis,* consistent with the present findings. These results reinforce the potential of combining cover crops with biological treatments as a sustainable strategy for nematode management in soybean production.

It is noteworthy that, regardless of the successful association with cover plants, the biological agents studied here show potential for nematode control when applied alone. *Bacillus*, for instance, controlled *P. brachyurus* in soybean (Mazzuchelli et al., 2020; Araujo et al., 2023). Seed treatment with *B. subtilis* reduced the *P. brachyurus* reproduction factor by 88% after 60 days of cultivation (Oliveira et al., 2019). Bacteria belonging to the genus *Bacillus* have varied nematode control mechanisms, including antibiosis, formation of a metabolically active biofilm in the rhizoplane, competition for penetration sites, and alteration of rhizosphere composition. These microorganisms also promote plant growth and induce plant defense responses (Hu et al., 2017; Hashem et al., 2019).

Similarly, previous research reported the efficiency of *P. lilacinum* + *T. harzianum* in controlling P. brachyurus in soybean (Dias-Arieira et al., 2018). Greenhouse and field experiments reduced nematode populations by 35.2 and 65.2%, respectively at 60 DAS (Dias-Arieira et al., 2018). *P. lilacinum* acts mainly by feeding on chitin, which is the predominant compound in nematode eggshells (Yang et al., 2015). The fungus can also infect adult females, degrading the embryo and reducing reproductive capacity, in addition to modifying nematode development and producing toxins that immobilize juveniles (Khan et al., 2006; Yang et al., 2015).

The cover plants used in the present study are classified as resistant or antagonistic crops (Amorim et al., 2019; Miamoto et al., 2021; Melo et al., 2023), except for *U. ruziziensis,* which is susceptible to *P. brachyurus* (Debiasi et al., 2016).

The results obtained for buckwheat agree with previous research (Melo et al., 2023). The plant is a feasible cover crop for reducing *P. brachyurus* populations in soybean rotation systems. This rustic plant has a short cycle, good adaptability to various types of soil, tolerance to high levels of acidity and aluminum saturation, and high absorption of phosphorus and potassium salts (Görgen et al., 2016; Melo et al., 2023). Additionally, secondary metabolites such as tannins, phenolic acids, and flavonoids accumulate in its seeds (Chitwood, 2002), which may contribute to nematode management.

Another interesting plant to use as a cover crop is pigeon pea, known to have a low reproduction factor for *P. brachyurus,* which can minimize nematode populations in succeeding soybean (Santana-Gomes et al., 2019). The cover crop is considered a soil decompressor, given its deep root system, with excellent water absorption and the possibility of recycling nutrients, especially nitrogen, at greater depths. Pigeon pea has high palatability and potential for forage production. It was deemed useful for recovering degraded pastures, whether in monoculture or rotation systems (L. C. Balbino et al., 2011).

Macrotyloma axillare 'Java' demonstrated good results in the current study combined or not with biological control agents. When combined with *U. ruziziensis,* it can potentially reduce *P. brachyurus* populations in succeeding soybean. In previous studies, Java was found to be a poor host to *P. brachyurus,* causing a reproduction factor of 1.87 to 2.12 (Miamoto et al., 2021).

It is interesting to note that few nematode control studies have investigated effects on soybean yield. The adoption of management practices by farmers does not depend solely on nematode control results but also on the potential yield gains. Here, it was found that Ur + pigeon pea, Ur + Java, Ur + buckwheat, and the cover crop mix combined with P. lilacinum + T. harzianum provided mean yield gains of 53.7, 59.0, 43.9, and 65.7%, respectively. The association of these crop systems with *B. subtilis* + *B. licheniformis* + *P. lilacinum,* except for Ur + pigeon pea, led to yield gains of 51.7 to 59.1% compared with the untreated control. These findings are consistent with previous studies reporting positive effects of *P. lilacinum* + *T. harzianum* on soybean yield (21% increase) (Dias-Arieira et al., 2018). The soybean yields observed here were relatively low, probably related to the high nematode populations in soybean roots (Tables 3, 4, and 5) and the limited space for root development, given the small experimental units.

Moreover, it is important to highlight that soybean cultivation in regions with sandy, low-fertility soils may intensify nematode-related issues. While nematode management strategies should focus on population control, future studies could investigate their relationship with soil recovery. This research is innovative, given that few studies combine biological and crop management methods for nematode control while addressing soybean yield improvement.

Studies on cover crops and biological control agents are incipient, and this management approach is highly complex. In practice, even when cultural and biological management strategies are combined, yield gains are not always achieved, and many aspects need further clarification. In this study, we did not explore in depth the mechanisms underlying the interaction between cover crops and biological control agents. Given the scarcity of literature on this topic, some results are difficult to corroborate.

Additionally, it is crucial to note that studies evaluating the effects of cover crops and biological control agents under controlled conditions, specifically in relation to yield, remain limited. Future research should explore how biological control agents interact with key cover crop species, enhancing the understanding of these associations and their potential benefits.

Conclusions

The combination of biological agents, such as *B. licheniformis* + *B. subtilis* + *P. lilacinum* and *P. lilacinum* + *T. harzianum,* with cultural management reduced *P. brachyurus* populations in soybean roots and surrounding soil. Regardless of biological treatment, cover crop treatments reduced nematode populations in soybean roots when compared with maize and *U. ruziziensis. U. ruziziensis* and bionematicides promoted soybean yield gains in the 2022 trial. In 2023, in light of the significant interaction effects, it was found that the combination of *U. ruziziensis* with other cover crops promoted the greatest soybean yield gains.

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