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Phytophatology/ Original Article

Management systems for nematode control in soybean fields in south-central Paraná, Brazil

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Abstract – The objective of this work was to evaluate the effects of winter crops and of soybean management systems on *Pratylenchus brachyurus* and *Helicotylenchus dihystera* populations, in a naturally infested site, in the south-central region of the state of Paraná, Brazil. The experiment was carried out during two crop years (2017/2018 and 2018/2019). Barley or black oat (winter crops) and soybean (summer crop) were treated with chemical or biological nematicides applied in the furrows or as seed treatment. Nematode reproduction on soybean was evaluated at 45 and 90 days after sowing (DAS). Soybean yield and 1,000-grain weight were also determined. The principal component analysis (PCA) of 2017/2018 showed a positive correlation between *P. brachyurus* and *H. dihystera* reproduction with barley/soybean + cadusafos, barley/soybean + abamectin, and barley/soybean + *Bacillus* spp., at 45 DAS, which shows that these treatments favored initial nematode reproduction. In the 2018/2019 crop year, the untreated barley/soybean, barley/soybean + abamectin, and black oat/soybean + abamectin systems favored the increase of 1,000-grain weight. The chemical control reduced *P. brachyurus* reproduction in both crop years. Black oat and the treatments with abamectin of winter and summer crops control *P. brachyurus* and increase soybean yield. However, the results are not conclusive for *H. dihystera* management.

Index terms: *Helicotylenchus dihystera*, *Pratylenchus brachyurus*, biological nematicide, chemical nematicide, winter crop.

Sistemas de manejo para o controle de nematoides em soja no centro-sul do Paraná, Brasil

Resumo – O objetivo deste trabalho foi avaliar o efeito de culturas de inverno e dos sistemas de cultivo de soja sobre populações de *Pratylenchus brachyurus* e *Helicotylenchus dihystera*, em área naturalmente infestada, na região centro-sul do Paraná, Brasil. O experimento foi realizado em dois anos agrícolas (safras 2017/2018 e 2018/2019). Cevada ou aveia-preta (culturas de inverno) e soja (cultura de verão) foram tratadas com nematicidas químicos e biológicos, aplicados aos sulcos ou em tratamentos de sementes. A reprodução dos nematoides em soja foi avaliada aos 45 e 90 dias após a semeadura (DAS). A produtividade da soja e a massa de 1.000 grãos também foram determinadas. A análise de componentes principais (PCA) da safra 2017/2018 mostrou correlação positiva entre a reprodução de *P. brachyurus* e *H. dihystera* e cevada/soja + cadusafos, cevada/soja + abamectina e cevada/soja + *Bacillus* spp., aos 45 DAS, o que mostra que estes tratamentos favoreceram a reprodução inicial dos nematoides. Na safra 2018/2019, os sistemas cevada/soja sem tratamento, cevada/soja + abamectina e aveia-preta/soja + abamectina proporcionaram aumento da massa de 1.000 grãos. O controle químico reduziu a reprodução de *P. brachyurus* nas duas safras. A aveia-preta e os tratamentos com abamectina das culturas de inverno e verão controlam *P. Brachyurus* e aumentam a produção de soja. No entanto, os resultados não são conclusivos quanto ao manejo de *H. dihystera*.

Termos para indexação: *Helicotylenchus dihystera*, *Pratylenchus brachyurus*, nematicida biológico, nematicida químico, cultura de inverno.

Introduction

Agricultural management systems directly interfere with nematode populations in the crop field (Debiasi et al., 2016). Management practices are known to vary greatly across Brazilian regions. In the southern part of the country, for instance, no-till systems are predominant and well-consolidated. Soybean [*Glycine max* (L.) Merr.] is typically grown in the summer, followed by a second soybean crop or a winter crop. In colder regions, such as in south-central Paraná state, maize is commonly grown as a second crop and wheat, oat, and barley as winter crops.

Regardless of the management system, crops included in succession programs may be susceptible to nematodes, especially *Pratylenchus brachyurus* (Godfrey, 1929) Filipjev & Shuurmans Stekhoven, 1941, an endoparasite reported in soybean, maize, and winter crops (Borges et al., 2010; Gardiano et al., 2012; Gonçalves et al., 2018; Matias et al., 2018), and *Helicotylenchus dihystera* (Cobb, 1892) Sher, 1961, characterized by wide host range (Ferraz & Brown, 2016) and recently reported as an endoparasite in soybean (Machado et al., 2019). Therefore, it is crucial that integrated nematode management strategies focus on crop protection, particularly for soybean. The major strategy for crop protection is to apply chemical or biological control agents in furrow or via seed treatment.

Studies confirmed the efficacy of chemical nematicides against plant-parasitic nematodes (Homiak et al., 2017). In addition to killing nematodes, most chemical nematicides exert a protective effect on plant roots, reducing nematode penetration during the residual period of the product, which may vary according to the active ingredient in the product, and to edaphoclimatic conditions. The most commonly used nematicides in soybean are abamectin, cadusafos, and thiodicarb (Higaki & Araujo, 2012; Homiak et al., 2017).

Currently, several biological products based on bacteria of the genus *Bacillus* are available in the market. Their mode of action involves the production of toxic metabolites that negatively affect nematode eggs and second-stage juveniles (J2), alteration of the rhizosphere microbial community, competition for penetration sites, colonization of the rhizoplane, and induction of plant defense against nematodes (Zheng et al., 2016). Other biological agents for nematode control are based on fungi, such as the widely studied specie *Purpureocillium lilacinum* (Thom) Luangsa-Ard et al., characterized as opportunistic, with saprophytic activity in soil, mainly parasitize nematode eggs and sedentary females (Dias-Arieira et al., 2018).

The integrated management of nematodes in soybean should include adequate cropping practices, such as crop rotation and/or succession, in combination with chemical or biological control methods (Favoreto et al., 2019). In southern Brazil, another aspect that needs to be considered is the false belief that nematodes do not cause problems in colder climates or soils rich in organic matter. Although yield losses in these regions may be related to the presence of nematodes, there is limited information on whether the use of adequate management practices can enhance soybean yields.

The objective of this work was to evaluate the effects of winter crops and of soybean management systems on *P. brachyurus* and *H. dihystera* populations, in a naturally infested site, in the south-central region of the state of Paraná, Brazil.

Materials and methods

The experiment was carried out in a soybean commercial field (25°32'34.67"S 51°31'23.26"W, at 1,093 m altitude), in the Entre Rios district, municipality of Guarapuava, in the state of Paraná, Brazil. According to the Köppen-Geiger's classification, the climate is Cfb (humid subtropical), without dry season, and with temperate summer and very cold winter; its annual means are 16.8°C temperature and e 1960 mm precipitation. The soil is classified as Latossolo Vermelho distrófico típico, according to the Brazilian system of soil classification (Santos et al., 2018), i.e., an Oxisol, with a very clayey texture.

A randomized complete block design with four replicates was used. The experimental units consisted of 3 m wide, 7.5 m long plots , totaling 22.5 m^2. Winter crops, barley (*Hordeum vulgare* L.) or black oat (*Avena strigosa* Schreb.) was sown at a 0.17 m row spacing, and soybean at 0.45 m row distance. One row from each side and 0.5 m borders on each end of the plot were not sampled to minimize border effects.

The treatments consisted of cropping systems and biological or chemical treatments (Table 1). Plots were cropped with barley 'Danielle', or black oat 'IAPAR 61 (Ibiporã)' in the winters, and with soybean 'DMario 58i' in the 2017/2018 summer, and soybean 'Produza IPRO' in the 2018/2019 summer.

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At the time of winter crop sowings, plots were fertilized with 500 kg ha^{-1} N-P_2O_5-K_2O 08-30-20 fertilizer + micronutrients FTe BR12 (Compass Minerals, Sumaré, SP, Brazil). Liming had been performed two years before the beginning of the experiment, using 3 Mg ha^{-1} dolomitic limestone (90% total neutralizing power). Lime and fertilizer applications were applied according to standard procedures adopted by the farm operator.

The experiment lasted two cropping years, including the 2017 winter, 2017/2018 summer, 2018 winter, and 2018/2019 summer seasons. The average minimum, mean, and maximum air temperatures were respectively: 10.4, 15.2, and 21.7°C during the 2017 winter; 14.5, 19.0, and 25.4°C during the 2017/2018 summer; 9.4, 14.3, and 20.7°C during the 2018 winter; and 15.2, 19.5, and 25.9°C during the 2018/2019 summer. Minimum, average, and maximum soil temperatures were respectively as follows: 15.3, 16.1, and 17.1°C in the 2017 winter; 21.0, 21.5, and 22.1°C in the 2017/2018 summer; 16.0, 16.2, and 16.6°C in the 2018 winter; and 21.6, 22.0, and 22.4°C in the 2018/2019 summer. The cumulative rainfall was 622 mm in the 2017 winter, 1,969 mm in the 2017/2018 summer, 586 mm in the 2018 winter, and 1,284 mm in the 2018/2019 summer. Data were obtained from the RADAR meteorological station of the Fundação Agrária de Pesquisa Agropecuária.

Table 1. Management systems applied in the winters of 2017 and 2018, and in the summers of the 2017/2018 and 2018/2019, for the control of *Pratylenchus brachyurus* and *Helicotylenchus dihystera* in soybean (*Glycine max*), in the municipality of Guarapuava, in the state of Paraná, Brazil.

Treatment	Winter	Summer
1	Barley	Soybean
2	Barley	Soybean + cadusafos
3	Barley	Soybean + *P. lilacinum*
4	Barley	Soybean + abamectin
5	Barley	Soybean + *Bacillus* spp.
6	Black oat	Soybean + cadusafos
7	Black oat	Soybean + *P. lilacinum*(1)
8	Black oat	Soybean + abamectin
9	Black oat	Soybean + *Bacillus* spp.(2)
10	Barley + cadusafos	Soybean + cadusafos
11	Barley + *P. lilacinum*	Soybean + *P. lilacinum*
12	Black oat + cadusafos	Soybean + cadusafos
13	Black oat + *P. lilacinum*	Soybean + *P. lilacinum*

(1)*Purpureocillium lilacinum: P. lilacinum + Trichoderma harzianum* + Moss. (2)*Bacillus* spp.: *B. subtilis + B. licheniformis*. Cadusafos and *P. lilacinum* were applied in furrows, whereas abamectin and the *Bacillus* species were applied via seed treatment.

Two biological nematicides were evaluated, one with *Purpureocillium lilacinum* and the other with *Bacillus* spp. The treatment with *P. lilacinum* consisted of *P. lilacinum* Pae-10 containing 7.5×10^{9} colony-forming units (CFU) kg^{-1} + *Trichoderma harzianum* Rifai IBLF006 containing 1×10^{10} CFU kg^{-1} + Moss (Nemat + Ecotrich WP + Pick Up Moss (Ballagro, Bom Jesus dos Perdões, SP, Brazil) at 0.1, 0.15, and 0.2 kg ha^{-1} product, respectively, with 500 L ha^{-1} in-furrow application of. It is important to note that *T. harzianum* (fungicide) and Moss (organomineral fertilizer) were included in the experiment as a company recommendation commonly adopted by producers in the region. The *Bacillus* spp. treatment consisted of *Bacillus subtilis* (Cohn) FMCH002 + *Bacillus licheniformis* (Weingmann) Chester FMCH001 (both bacterial products containing a minimum of 1.0×10^{11} CFU g^{-1} and 200 g kg^{-1}) at 100 g 100 kg^{-1} seed, and 500 mL 100 kg^{-1} volume applied via seed treatment. Two chemical agents were used, cadusafos and abamectin. Cadusafos (200 g L^{-1}, Rugby 200 CS, FMC Agrícola, Campinas, SP, Brazil) was applied in furrows at 4 L ha^{-1} and 100 L ha^{-1} rate. Abamectin (500 g L^{-1}, Avicta 500 FS, Syngenta, Holambra, SP, Brazil) was used as seed treatment at 125 mL 100 kg^{-1} seed, and 400 mL 100 kg^{-1} seed rate.

Samples for nematode analysis were collected in the summer, before the beginning of the experiment, and at 45 and 90 days after sowing (DAS), to determine all phytonematodes present in the samples. In the first collection, only soil samples were taken, whereas, at 45 and 90 DAS, soybean roots and rhizosphere soil were collected with a shovel. Five subsamples were taken from each plot and combined to form a composite sample. Soil and roots were separated in the laboratory. Roots were washed and homogenized, and 10 g aliquots were subjected to nematode extraction according to Coolen & D'Herde (1972). Soil was homogenized, and nematodes were extracted from 100 cm^{3} aliquots, following the method of Jenkins (1964). Counting and identification of plant-parasitic nematodes were performed using a Peters' chamber under a light microscope at 100X magnification. The total number of nematodes in roots and soil, at

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45 and 90 DAS, was divided by the initial population in the soil, to obtain the reproduction factor (RF) (Oostenbrink, 1966).

At the end of each crop cycle, soybean was harvested, and 1,000-grain weight (TGW) was determined. Data were subjected to the univariate analysis, at 5% probability and, when significant, means were compared by the Scott-Knott's test, at 5% probability, using the Sisvar software (Ferreira, 2011). The principal component analysis (PCA) was used to investigate relationships between *P. brachyurus* RF, *H. dihystera* RF, TGW, and cropping treatments at 45 and 90 DAS. The effects of winter crop species (barley or black oat), type of control (chemical or biological), and number of applications (summer or winter and summer) on the studied variables were compared by the Student's t-test, at 5% probability, using the Statistica software version 10.0 (TIBCO Software Inc., Palo Alto, CA, USA).

Results and Discussion

The experimental site was infested with *P. brachyurus* and *H. dihystera* only. PCA of 2017/2018 crop data showed that, at 45 DAS, the RF of *P. brachyurus* and *H. dihystera* correlated positively with each other and with barley/soybean + cadusafos (T2), barley/soybean + abamectin (T4), and barley/soybean + *Bacillus* spp. (T5). Thus, T2, T4, and T5 favored the nematode development (Figures 1 A and B).

At 90 DAS, the behavior of *P. brachyurus* RF was the same as that observed at 45 DAS. However, *H. dihystera* RF correlated positively with barley + *P. lilacinum* / soybean + *P. lilacinum* (T11) and black oat + *P. lilacinum* / soybean + *P. lilacinum* (T13). TGW showed a negative correlation with *P. brachyurus* RF, and a positive correlation with black oat/soybean + abamectin (T8) (Figures 1 A and B).

In 2018/2019, the RF values of *P. brachyurus* at 45 and 90 DAS showed a positive correlation with each other and with black oat/soybean + cadusafos (T6) (Figure 2). The RF of *H. dihystera* at 90 DAS correlated positively with barley/soybean + cadusafos (T2), barley/soybean + *P. lilacinum* (T3), barley/soybean + *Bacillus* spp. (T5), black oat/soybean + *Bacillus* spp. (T9), barley + cadusafos/soybean + cadusafos (T10), and black oat + cadusafos/soybean + cadusafos (T12). TGW correlated negatively with *H. dihystera* RF at 90 DAS. Moreover, untreated barley/soybean (T1), barley/soybean + abamectin (T4), and black oat/soybean + abamectin (T8) enhanced the TGW (Figure 2).

The result comparisons for 2017/2018 showed that the cropping treatments exerted significant effects on *P. brachyurus* RF (Figure 3 A), but not on *H. dihystera* RF (Figure 3 B). The RF of *P. brachyurus* at 45 DAS was not lower in cropping treatments than in the control. However, at 90 DAS, RF was reduced under black oat/soybean + cadusafos (T6), black oat/soybean + abamectin (T8), barley + cadusafos / soybean + cadusafos (T10), barley + *P. lilacinum* / soybean + *P. lilacinum* (T11), black oat + cadusafos / soybean + cadusafos (T12), and black oat + *P. lilacinum* / soybean + *P. lilacinum* (T13). The average reduction of these cropping treatments was approximately 55% compared to the control.

The greater dispersion of treatments in the biplot of the first agricultural year (2017/2018) facilitated the identification of differences between treatments and their effects on the studied variables (Figure 1 A and B). However, for 2018/2019, treatments were plotted close to each other, impairing the analysis of correlations or differences between variables (Figure 2 A and B). Because the experimental site was the same, these differences between years were likely due to climatic variations. The higher temperatures recorded in the second year might have favored the nematode development, conferring metabolic advantage to nematode (McSorley, 2003). Rainfall was higher in the first experimental year (685 mm more than in the summer of the second year), which might have affected the soybean physiological activity (Figure 3).

Pratylenchus brachyurus showed the lowest RF at 45 DAS, when soybean was grown in succession to black oat (Figure 4 A). Black oat seemed to have the lowest *P. brachyurus* RF, which corroborates previous studies showing that black oat has potential to control this nematode species (Borges et al., 2010). Black oat 'IAPAR 61 (Ibiporã)', used in the current study showed resistance to nematodes (RF = 0.26), confirming the findings by Gabriel et al., (2019). In a naturally infested site, black oat 'IAPAR 61 (Ibiporã)' proved to be one of the best winter cereals, both as a single crop and as an intercrop with forage turnip (*Raphanus sativus* L. var. *oleiferus* Metzg.), reducing *P. brachyurus* reproduction on the following maize crop (Chiamolera et al., 2012). We could not find any recent study on the susceptibility of Brazilian barley genotypes to *P. brachyurus*.

The type of control (whether chemical or biological) significantly influenced nematode populations in both experimental years. Chemical products were more efficient (next to 38%) than biological agents in the reduction of *P. brachyurus* RF on soybean (Figure 4 B). Some studies have reported the efficiency of chemical products on the control of lesion nematodes in soybean (Homiak et al., 2017). Chemical products make active

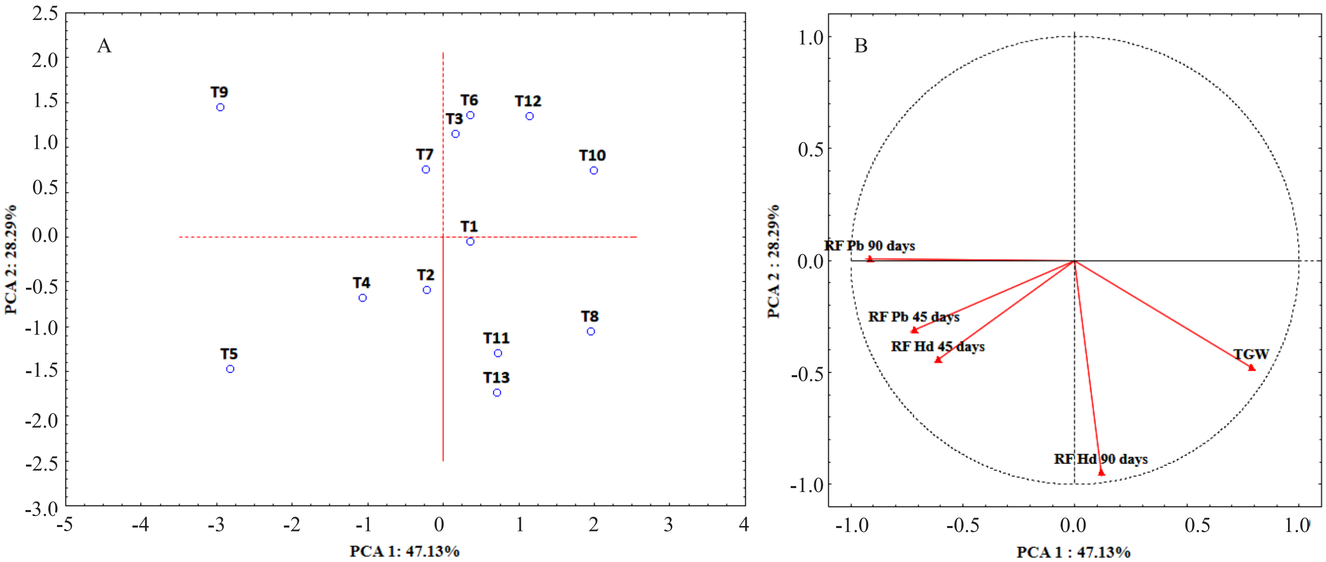

Figure 1. Principal component analysis (PCA) of cropping treatments (see Table 1): A, score plot; B, loading plot; RF, reproduction factor of *Pratylenchus brachyurus* (Pb) and *Helicotylenchus dihystera* (Hd), at 45 and 90 days after sowing; and TGW, 1,000-grain weight of soybean (*Glycine max*), at the end of the 2017/2018 crop season, in the municipality of Guarapuava, in the state of Paraná, Brazil.

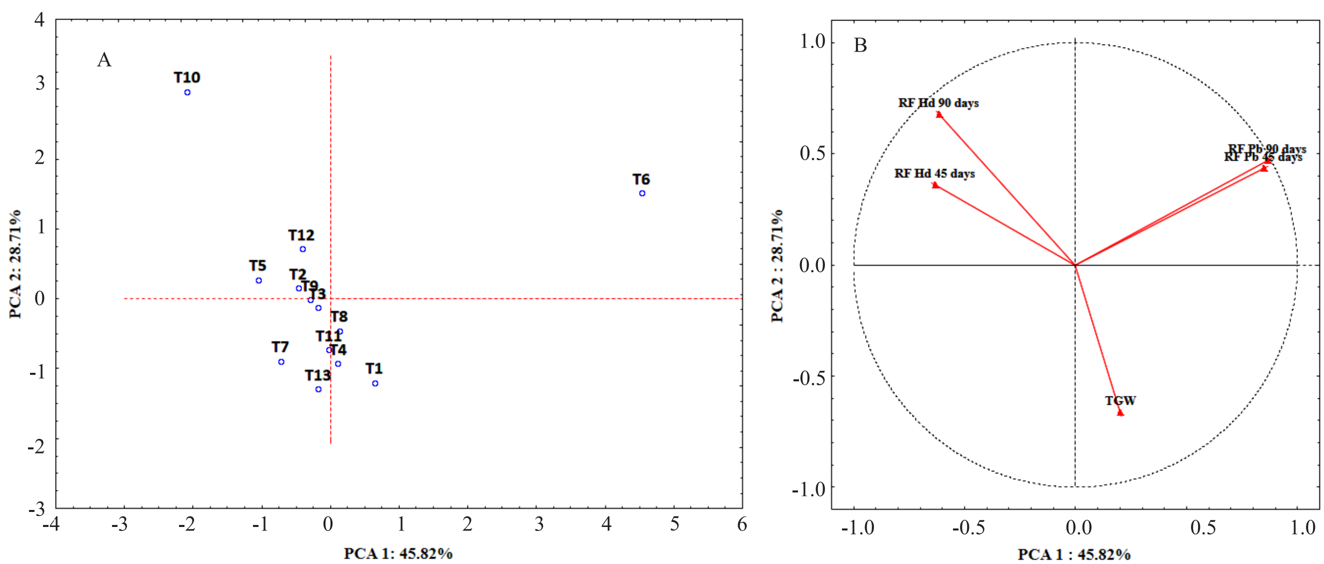

Figure 2. Principal component analysis (PCA) of cropping treatments (see Table 1), represented by: A, score plot; B, loading plot; RF, reproduction factor of *Pratylenchus brachyurus* (Pb) and *Helicotylenchus dihystera* (Hd), at 45 and 90 days after sowing; and TGW, 1,000-grain weight of soybean (*Glycine max*), at the end of the 2018/2019 crop season, in the municipality of Guarapuava, in the state of Paraná, Brazil.

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ingredients readily available, which represents an advantage over biological agents that require a given period to colonize the rhizoplane or rhizosphere, before acting against nematodes. A previous study showed that chemical agents were more effective than biological ones in reducing *P. brachyurus* populations in soybean, for up to 60 days after inoculation (Oliveira et al., 2019); however, the same study showed that, at 120 days after inoculation, biological agents had a more pronounced effect. This finding can be explained by the fact that chemicals have a relatively short residual period, which results in a decrease of efficiency with time (Vitti et al., 2014). However, biological agents tend to persist in the soil and are stimulated by the presence of nematodes, root exudates, and/or organic matter (Li et al., 2019).

Despite these differences, the efficiency of biological control is indisputable. Previous field and greenhouse experiments showed that *P. lilacinum* + *T. harzianum* + Moss were efficient in the control of *P. brachyurus* in soybean (Dias-Arieira et al., 2018). Similarly, *Bacillus* spp., applied alone or in combination, was effective in decreasing lesion nematode populations (Miamoto

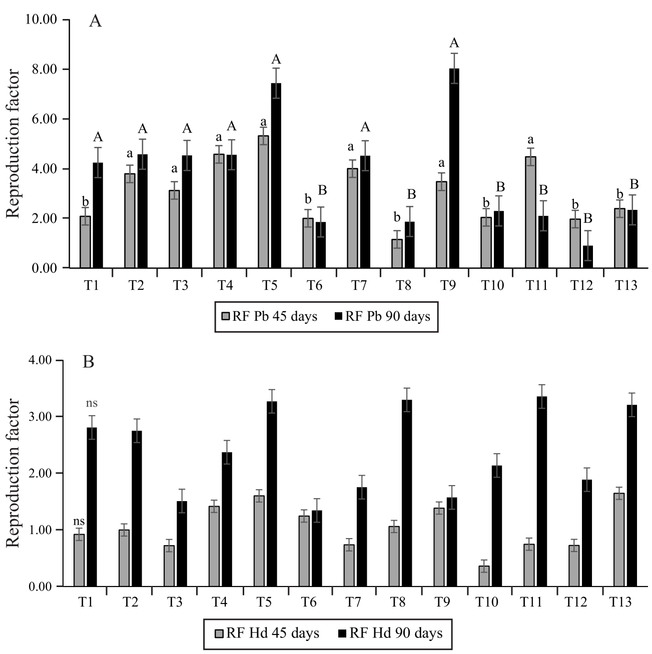

Figure 3. Reproduction factor (RF) of the nematode species on soybean (*Glycine max*), at 45 and 90 days after sowing, in the 2017/2018 season: A, *Pratylenchus brachyurus* (Pb); and B, *Helicotylenchus dihystera* (Hd). Different lowercase letters indicate significant differences between treatments (see Table 1) at 45 days after sowing, and different uppercase letters denote significant differences between treatments at 90 days after sowing, by the Scott-Knott's test, at 5% probability. Error bars represent the standard error between periods within treatments. [ns]Nonsignificant.

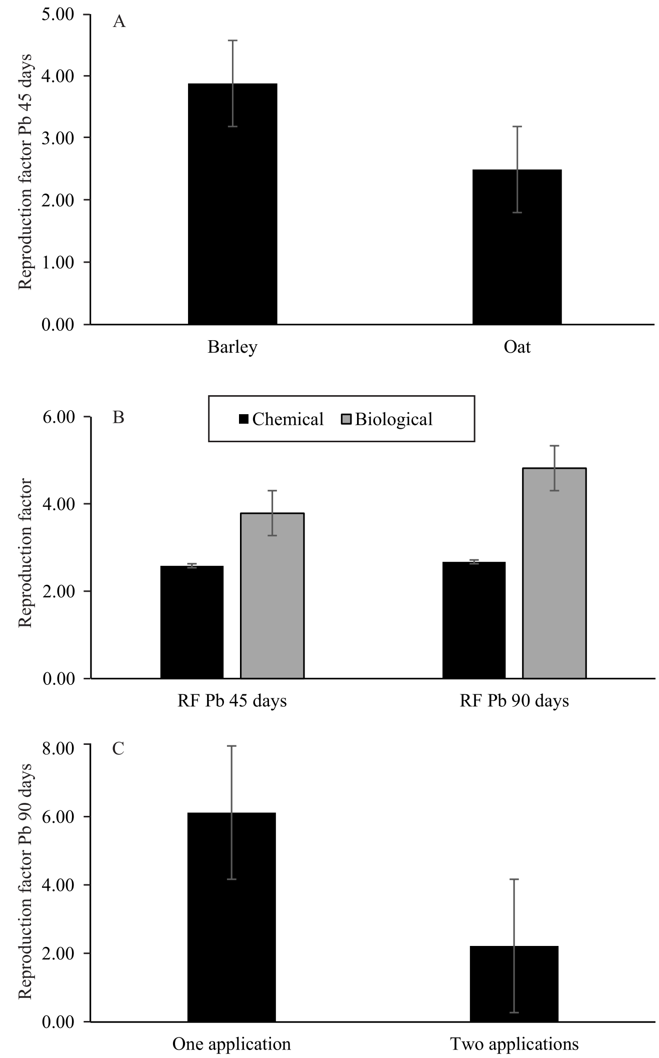

Figure 4. Reproduction factor (RF) of *Pratylenchus brachyurus* (Pb) on soybean, at 45 and 90 days after sowing, in the 2017/2018 season, compared between independent groups. A, comparison between winter crops: barley or black oat; B, comparison between chemical (cadusafos and abamectin) and biological agents (*Purpureocillium lilacinum* + *Trichoderma harzianum* + Moss), or *Bacillus subtilis* + *B. licheniformis*); C, comparison between one application (summer) and two applications (winter and summer) of the biological or chemical products. Means were compared by the Student's t-test, at 5% probability. Error bars represent the standard error of the mean.

et al., 2017; Oliveira et al., 2019), with an efficiency comparable to that of chemical agents (Higaki & Araujo, 2012). It is important to note that edaphoclimatic factors can directly influence the ability of biological agents to control nematodes (Abd-Elgawad & Askary, 2020). The soil of the study site (Guarapuava, PR) had good levels of organic carbon (32.32 g dm^{-3}) and organic matter (5.57%), which indicates that there was a high biological diversity in it (Don et al., 2017) and, consequently, a greater competition between natural soil inhabitants and those introduced for biocontrol. Although rhizosphere colonization is paramount for effective biological nematode control (Siddiqui & Shaukat, 2003), it is not known which factors affect the competence of the rhizosphere in the interaction between biological control agents and nematodes.

Double (summer and winter) applications rather than a single one (summer only) of treatments resulted in a higher *P. brachyurus* control (Figure 4 C). It is known that nematicides do not completely eliminate nematode populations. Chemical and biological agents reduce nematode populations by 60–80% on average (Miamoto et al., 2017; Dias-Arieira et al., 2018; Oliveira et al., 2019). The surviving nematodes can penetrate roots and multiply throughout the crop cycle. Lesion nematodes deposit eggs inside roots (Ferraz & Brown, 2016). Thus, lesion nematodes can complete successive cycles without being exposed to external agents. Infected roots remain as the main source of inoculum in soil, allowing of the survival of lesion nematodes under adverse conditions (Ribeiro et al., 2020). Nonetheless, root-knot nematodes deposit their eggs on the outer surface of roots, that is, in the soil (Ferraz & Brown, 2016), causing subsequent generations to be continuously exposed to chemical and biological agents present in the rhizosphere and rhizoplane.

Therefore, to guarantee low levels of lesion nematodes throughout the crop cycle, it is crucial that the initial penetration be efficiently controlled. This can explain the superior performance of chemical agents over biological ones, as the latter require a longer period to become established in soil. Treatment reapplication seems to be an effective strategy for an enhanced control of *P. brachyurus*; this practice should be better investigated and stimulated.

Regarding *H. dihystera* control, the PCA of the second experimental year showed a negative correlation between *H. dihystera* RF and TWG, but no significant differences were found between treatments by the analysis of variance. Studies on the parasitism and epidemiology of *H. dihystera* in tropical countries are still scarce, especially because this species generally occurs in mixed populations. Nevertheless, *H. dihystera* has a wide range of hosts (Ferraz & Brown, 2016; Favoreto et al., 2019) and may act as a migratory endoparasite of soybean (Machado et al., 2019), although it is more frequently found in the soil (Leiva et al., 2020).

No significant differences for yield were observed between treatments. In fact, the complexity of interactions involved in nematode management makes it difficult to identify differences for yield parameters (Dias-Arieira et al., 2018). Black oat/soybean + cadusafos, black oat/soybean + *P. lilacinum*, and black oat/soybean + abamectin afforded a yield increase of 34.8, 18.0, and 64.2 kg ha^{-1} in 2017/2018, and 78.6, 102.0, and 54.0 kg ha^{-1} in 2018/2019, respectively. These findings indicate that black oat may increase soybean yield, as evidenced by the PCA results for 2017/2018.

Integrated nematode management systems are complex and require careful planning, particularly in areas infested with nematodes that have a wide range of hosts, such as *P. brachyurus*. This study showed that the application of chemical or biological control agents in the winter and the correct choice of rotation crops are essential for nematode control and crop productivity.

Conclusions

1. Black oat (*Avena strigosa*) is the best winter crop to control *Pratylenchus brachyurus* and enhance soybean (*Glycine max*) yield in the south-central region of Paraná.

2. Chemical treatment with abamectin nematicide of winter crop and soybean is crucial for the control of *P. brachyurus* in soybean.

3. The results are not conclusive for the *Helicotylenchus dihystera* management.

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