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Genetic, chemical and biological management strategies of control of *Pratylenchus brachyurus* in soybean

Estratégias de manejo genético, químico e biológico de Pratylenchus brachyurus em cultivo de soja

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ABSTRACT: Brazil is the second largest producer of soybeans in the world. However, infestations of *P. brachyurus* have been decreased the gains for higher productivity and it may cause damages up to 50% for this production trait. We aimed with this study to evaluate the effectiveness of chemical and biological agents on for *P. brachyurus* control in two soybean cultivars, under three modes of application. A completely randomized block design was used in the factorial scheme 4 x 3 x 2 + 2, with 3 replications. The treatments consisted of a combination of four control agents, two chemical (abamectin, imidacloprid + thiodicarb) and two biologicals (*Bacillus subtilis* and *Trichoderma atroviride*); three ways of application (seed treatment, application in the seeding furrow and spraying at stage V1), two cultivars ('Anta 82' and 'Brasmax Desafio') with different reproductive factors, and one control for each cultivar, with inoculation of *P. brachyurus* and without any control agent (negative control). Each plot received 1000 *P. brachyurus* individuals and were evaluated at 60 days after inoculation. All control agents can be indicated to manage *P. brachyurus*, *T. atroviride* and imidacloprid + thiodicarb are the most efficient ones. The ways of application do not influence the performance of the control agents for reducing nematodes.

Keywords: Alternative control. *Glycine max*. Phytonematode.

RESUMO: O Brasil é o segundo maior produtor de soja no mundo. Contudo, infestações de *P. brachyurus* têm prejudicado a obtenção de maiores produtividades e podem causar danos de até 50% dessa variável produtiva. Objetivou-se com o presente estudo avaliar a eficácia de agentes químicos e biológicos no controle de *P. brachyurus* em duas cultivares de soja, sob três modos de aplicação. Foi utilizado o delineamento inteiramente casualizado no esquema fatorial 4 x 3 x 2 + 2, com 3 repetições. Os tratamentos foram constituídos pela combinação de 4 agentes de controle: 2 químicos (abamectina, imidacloprido + tiodicarbe) e dois biológicos (*Bacillus subtilis* e *Trichoderma atroviride*); 3 modos de aplicação (tratamento de sementes, aplicação no sulco de semeadura e pulverização no estádio V1), duas cultivares ('Anta 82' e 'Brasmax Desafio') com diferentes fatores de reprodução, e 1 testemunha para cada cultivar, com inoculação de *P. brachyurus* e sem a aplicação de agente de controle. Todas as parcelas foram inoculadas com 1000 indivíduos de *P. brachyurus* e foram avaliadas aos 60 dias após a inoculação. Todos os agentes de controle são indicados no manejo de *P. brachyurus*, *T. atroviride* e Imidacloprido são mais eficientes. Os modos de aplicação não influenciam no desempenho dos agentes para redução dos nematoides.

Palavras-chave: Controle alternativo. Fitonematoides. *Glycine max*.

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INTRODUCTION

Soybean is one of the bases of Brazilian agrobusiness, with impressive growth of the total amount harvested. However, along with its expansion, there have been increase of infestation by pathogenic agents and other pests (MACHADO; AMARO; SILVA, 2019). According to (ALLEN *et al.,* 2017), soybean diseases may decrease yield by 28% in the fields. Among these, phytonematodes have increasingly causing economic damage.

In Brazil, the nematodes of the genus *Pratylenchus* stands out, known for causing root lesions in plants and, consequently, losses in productivity (GOULART, 2008; NUNES; MONTEIRO; POMELA, 2010; DIAS-ARIEIRA *et al*., 2018). Some cultivation practices, such as no-tillage planting system and the cultivation on sandy soils, favor the spread and establishment of huge populations of this pathogen, resulting in higher losses (CRUZ; ASMUS; GARCIA, 2020). Nematodes of the species *P. brachyurus* have high mobility after penetrate the root system of host plants, feeding of the root cortex cells, causing damage and browning of parasitized site, besides of formation of wrinkled leaves, restricting the formation of pods, in addition to the early plant ripening, generating damage that varies between 30 to 50% depending on the intensity of the infestation (GOULART, 2008; DIAS *et al*., 2010; DIAS-ARIEIRA *et al*., 2018; CASTANHEIRA *et al*., 2020).

In infested areas, aiming to the management of populations of *P. brachyurus* below the economic threshold, many control strategies are recommended, such as chemicals, crop rotation, biologicals, cultural traits and the use of resistant cultivars (LOPES *et al.,* 2017; BERNARDES et *al*., 2019; OLIVEIRA *et al*., 2019). The use of chemical products has become an effective tool for areas with nematodes, however, their isolated applications, apart from an integrated management, only decrease populations for a short period, requiring constant applications, which leads to dependence in areas of high infestation, besides of being considered highly toxic to the environment.

Thus, it is necessary the constantly search for new strategies of integrated management and, in this sense, several researches have reported the promising potential of biological management of nematodes through protozoa, fungi and bacteria (MÁSCIA, 2017; DIAS-ARIEIRA *et al.,* 2018; OLIVEIRA *et al.,* 2019; HUSSAIN *et al*., 2020; LOPES *et al*., 2020; NADEEM *et al*., 2020; PACHECO *et al*., 2020; OLIVEIRA *et al*., 2021). The plant growth-promoting rhizobacteria (PGPR) have proved to be efficient and viable. These microorganisms can stimulate induced systemic resistance (ISR) in plants, through the secretion of compounds released inside the rhizosphere. For this reason, bacteria from rhizospheres of many plants are often isolated for biological control studies, pointing out the species *Bacillus subtilis* and *B. methylotrophicus* (MEDEIROS *et al.,* 2019).

The use of fungi of the genus *Trichoderma* is also a management strategy, since it is antagonist to nematodes, due to their ability to degrade chitin, controlling, then, the nematodes and acting on the eggs and juveniles, which decreases soil populations (KATH *et al*., 2017; LOPES *et al*., 2020; PACHECO *et al*., 2020).

Considering the integrated disease management strategies, the use of resistant cultivars may help the control of nematodes populations in cultivated areas, in association with the application of chemical and/or biological agents. Currently, there are several soybean cultivars with different levels of resistance, and it is up to the farmer to choose the most appropriate one to his reality, and should prefer, whenever possible, for those with the highest level of resistance for areas of higher infestation (MACHADO; ARAÚJO FILHO, 2016).

In face of the serious problems caused by nematodes in agricultural crops, studies that seek new control methods are necessary, aiming to reduce the impact of chemicals on the environment. Thus, we aimed to evaluate the efficacy of controls chemical and biological of the nematode *P. brachyurus* via three ways of application in two soybean cultivars with different levels of resistance.

2 MATERIAL AND METHODS

The experiment was carried out in a greenhouse in the Mato Grosso State University, in Alta Floresta city, located at the coordinates West 55° 30' and South 9° 00'. According to the classification by Köppen e Geiger (2017), the climate of the region is tropical (Am) with greater pluviosity in summer than in winter, with an average temperature of 25.4 ºC and an average annual rainfall of 2,281 mm.

A completely randomized design was used in a factorial scheme 4 x 3 x 2 + 2, with 3 replications. The treatments were composed by combination of four control agents, 2 chemicals (abamectin, imidacloprid + thiodicarb) and 2 biologicals (*B. subtilis* and *T. atroviride*), with 3 ways of application (seed treatment, seeding furrow and pulverization at the V1 stage), 2 cultivars ('Anta 82' and 'Brasmax Desafio'), and 2 controls treatments for each cultivar with inoculation of *P. brachyurus* and without any control agent (negative controls), totaling 26 treatments.

The experimental units were composed of pots with a capacity of 8 dm^{-3}, which were filled with Dystrophic Yellow-Red Latosol (OXISOL), collected in the 0-0.20 m soil layer. Fertilization and soil fertility correction followed the recommendation of Malavolta (1981). Plots were filled with substrate composed by soil and sand in proportion 3:1, previously sterilized in autoclave for 2 hours at 121° C, at 1.0 atm pressure.

The inoculum containing eggs, juveniles and adults of *P. brachyurus* was provided by the Laboratory of Nematology of Mato Grosso Federal University (UFMT) in Barra do Garças city, from soil and soybean plants collected in infected areas. The nematodes were then extracted from samples of soil by the method of Jenkins (1964), and from roots by the method of Coolen and d'Herde (1972). After the extractions, *P. brachyurus* extracted population was quantified with aid of Peters counting chamber (HANDOO; GOLDEN, 1989; TIHOHOD, 1997). Then, the volume of suspension equivalent to 1,000 individuals (juveniles and adults) was stipulated, and, thereafter applied in each plot.

The inoculants containing *B. subtilis* and *T. atroviride* were used in proportion of 300 ml for each 50 kg of seeds. The suspension of *T. atroviride* was standardized to the concentration of 2,5 x 10^9 viable conidia mL^{-1}.

Abamectin was applied in the proportion of 60 ml to 50 kg of seeds, using the commercial product Avicta ® 500 FS. Imidacloprid + thiodicarb was applied in the proportion of 300 ml to 50 kg of seeds, using the commercial product Cropstar ®. All seeds were inoculated with *Bradyrhizobium japonicum* for the purpose of biological nitrogen fixation.

For treatments that consisted of furrow application, biological and chemical agents were applied on the seeds previously placed in the furrows, which were covered right after. For the treatments which received any control at the V1 stage, the application was performed simulating a mechanical spray volume of 200 L h^{-1}, using sprayer with constant pressure of CO_2.

The chemical and biological treatments were evaluated in two soybean varieties, selected according to the level of resistance to *P. brachyurus*, they are 'Anta 82' (moderately resistant) and ' Brasmax Desafio' (susceptible). Six seeds per pot were sown, and two days after emergence (DAE) roguing was performed, keeping only one plant per pot.

The plots were inoculated with 1,000 *P. brachyurus* individuals, with the aid of micropipette at 12 DAE. In each plot, nematode suspension was applied in three 2 cm deep holes in the soil, placed at 2 cm distant from plant stem. The holes were immediately covered right after inoculation, to prevent nematode desiccation (NUNES; MONTEIRO; POMELA, 2010). Irrigations were carried out manually, with use of watering can, always aiming to keep the soil moisture close to field capacity level.

At 60 days after inoculation (DAI) of nematodes, the following traits were evaluated: number of eggs and nematodes in 100 cm^3 of soil, number of eggs and nematodes in 10 g of roots and Reproduction Factor, following the recommendations of Oliveira *et al*. (2011).

P. brachyurus individuals in the soil for each plot were extracted and quantified from samples containing 100 cm^3 of soil, as recommended by Jenkins (1964). For root processing, these were washed and then, 10-gram sample was taken to proceed with the extraction. Later, roots sampled were cut into 2 cm pieces and processed in a blender for 30 seconds at low speed in a 0.5% sodium hypochlorite solution and, then carried to sifting, sedimentation and centrifugation (COOLEN; D'HERDE, 1972). Once the quantification of *P. brachyurus* was done, the value was extrapolated to the soil volume of each pot. Then, the final population (soil population + root population) and, therefore, the Reproduction Factor (RF) was calculated using the equation:

$$RF = \frac{Final\ Population\ (FP)}{Initial\ Population\ (IP)}$$

The data were subjected to the Analysis of Variance and the treatment averages were compared by the Scott-Knott at 5% of probability. The variables were transformed to $\sqrt{(x+0.5)}$. The analyzes were performed with the aid of the package ExpDes of the software R, of public domain (R DEVELOPMENT CORE TEAM, 2020).

3 RESULTS AND DISCUSSION

There were no significant effects of the ways of application for any of the variables evaluated, with no significant interaction between this source of variation and the others (Table 1). The absence of effects regarding the mode which nematicides may be applied has also been proved by Wilson et al. (2020) for chemical treatments.

Taking into account the nematode's need to parasitize the plant's rhizosphere as soon as seed germination occurs, it is pertinent to choose the method of application that protects the crop from the initial stages. In studies about the control of nematodes, Henning (2005) found that the most affordable method of application is seed treatment, since in addition to being efficient, it represents only 0.5% of the cost of crop planting. According to the present results, farmers can choose either application at seeds, furrow or spraying.

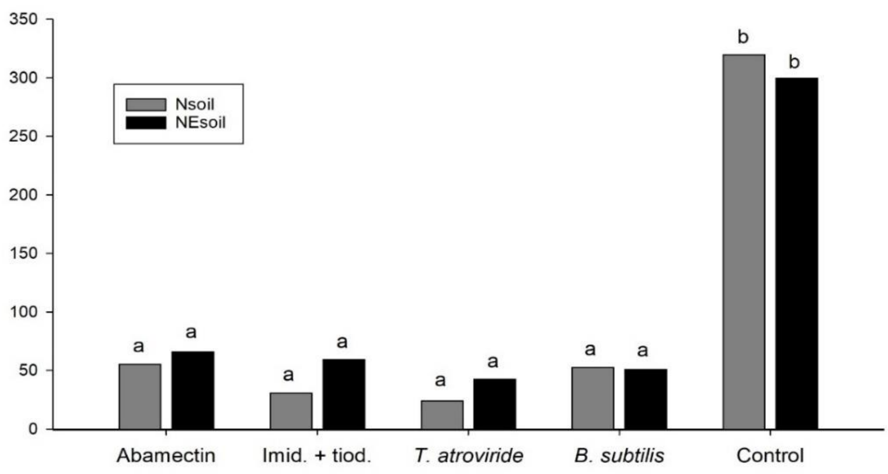
Table 1. Summary of analysis of variance (F values) for number of nematodes in the soil (Nsoil), number of eggs in the soil (NEsoil), number of eggs in the root (NEroot), number of nematodes in the root (Nroot) and reproduction factor (RF) of soybean plants submitted to different treatments for the control of *Pratylenchus brachyurus*. Alta Floresta – MT, 2018

	DF	Nsoil^z	NEsoil^z	NEroot^z	Nroot	RF
Agents	4	57.6**	195.0**	5.1**	37.9**	37.5**
Ways of application	2	1.0 ns	0.7 ns	0.0 ns	1.4 ns	0.9 ns
Cultivars	1	0.2 ns	3.2 ns	5.4 **	47.0**	42.4 **
Agents x Ways	8	0.2 ns	1.4 ns	0.9 ns	1.6 ns	0.5 ns
Agents x Cultivars	4	0.4 ns	1.4 ns	2.9**	26.3**	27.7**
Ways x Cultivars	2	0.3 ns	1.1 ns	1.1 ns	2.6 ns	0.6 ns
Agents x Ways x Cultivars	8	0.2 ns	0.5 ns	1.2 ns	2.3 ns	0.7 ns
CV%		44.3	25.1	44.4	29.4	11.86

** and ns – Means are significant at 1% and not significant by the F test, respectively. CV- Coefficient of variation. ^{z}Data transformed to the analyses in $\sqrt{(x+0.5)}$

Significant effects were observed for the control agents in all variables. There was also an influence of cultivars for NEroot, Nroot and RF, and this behavior was also observed for the interaction between agents x cultivars (Table 1).

Considering only the control agents, all treatments obtained statistically equal responses, but, more efficient than the control for Nsoil and NEsoil (Figure 1). In absolute values, the results obtained by the application of *T. atroviride* stand out. According to Oliveira et al. (2021), *Trichoderma* spp. has high biotechnological potential, efficient in the control of phytopathogens, especially those that inhabit the soil, such as *Sclerotinia*, *Fusarium* sp., *Phytophthora* sp., *Pythium* sp., *Rhizoctonia* sp. (HADDAD, 2014; NAWROCKA; SZCZECH; MAŁOLEPSZA, 2017) and phytonematodes (KATH *et al*., 2017; MÁSCIA, 2017; AMARAL *et al*., 2018; FRACETO *et al*., 2018; WOHLENBERG, 2018).



Means followed by same letter on the columns with the same color do not differ at the 5% level by the Scott-Knott test.

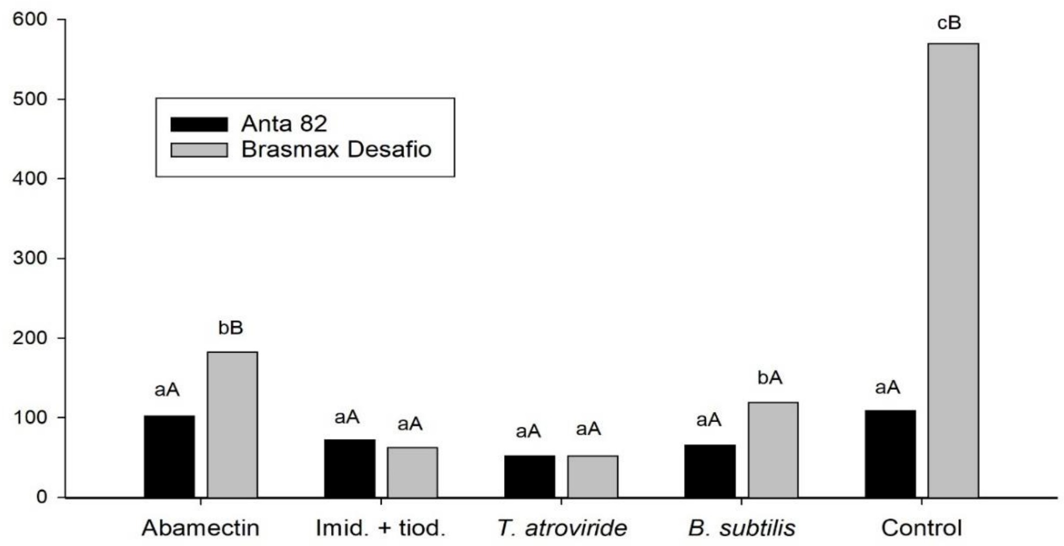
Figure 1. Average values of control agents of *P. brachyurus* for number of nematodes in soil (Nsoil) and number of eggs in soil (NEsoil) of soybean plants. Alta Floresta, MT, 2018.

The action of *T. atroviride* in the control of nematodes may occur through antagonism, competition, mycoparasitism, production of toxic metabolites or by the production of extracellular enzymes that degrade the egg chitin and cell walls of second-stage (IBRAHIM *et al*., 2020). So, its biocontrol activity is related to the synthesis of hydrolytic enzymes such as: chitinases, glucanases, proteases and nagases that degrade the cell wall of other organisms (SILVA; ULHOA, 2011). Therefore, the use of *Trichoderma* in the control of nematodes is effective, as it is a cheaper alternative that does not harm the environment, and may be applied in soybean cultivars susceptible to the root-lesion nematodes which, pointing out that such cultivars have higher productivity potential and low cost, comparing to the resistant ones.

The nematode control in both cultivars established by Imidacloprid + thiodicarb possibly occurred because it is a fast-acting contact nematicide, protecting the plant in the early stages of development, which corroborates with Wilson *et al*. (2020), Kubo, Machado and Oliveira (2012) and Bortolini *et al.* (2013), who were successful in decreasing *Rotylenchus reniformis* and *P. brachyurus* populations. In the same way, Homiak *et al*. (2017) highlighted the efficiency of those active principles for *P. brachyurus* control in roots, either by each one separately or combined into one application, even though they did not result in gains of productivity.

The efficiency of genetic factors for nematode management was detected. For 'Anta 82', there were no differences between the treatments and the control (Figure 2). This answer is similar to the results found by Araújo, Bragante and Bragante (2012) who, working with the soybean cultivar BRS 282, resistant to *Meloidogyne* spp., and applying *B. subtilis* and/or carbofuran, found no difference between treatments and the control for nematode management.

Genetic resistance of plants against phytopathogens is most desirable management method. According to Kamunya *et al*. (2008), it may, in some cases, dismiss the use of chemical or biological products, since a resistant genotype, in addition to having a low nematode reproduction factor, is environmentally the best option and low-cost strategy to the farmers. In fact, findings of Silva *et al*. (2020) have shown the availability of soybean cultivars resistant to *P. brachyurus* and *P. zeae*, in which the cultivar NA 5909 RG is highly resistant to both.



Means followed by the same lowercase letter between cultivars and uppercase among agents do not differ at the 5% level of significance by the Scott-Knott test.

Figure 2. Average values of number of nematodes in root (Nroot) of soybean plants for the significant interaction between agents and cultivars, aiming at the control of *Pratylenchus brachyurus.* Alta Floresta, MT, 2018.

In contrast, for the application of agents in the susceptible cultivar 'Brasmax Desafio', all treatments showed superior performance in comparison to the control. In this cultivar, all significant results are due to the effects of control agents, with emphasis for *T. atroviride* and Imidacloprid + Thiodicarb. Kath *et al*. (2017) using different populations of *Trichoderma* spp., found that there is effectiveness and variation in the management capacity of *P. brachyurus* depending on the isolate, showing genetic variability in this fungus, as well.

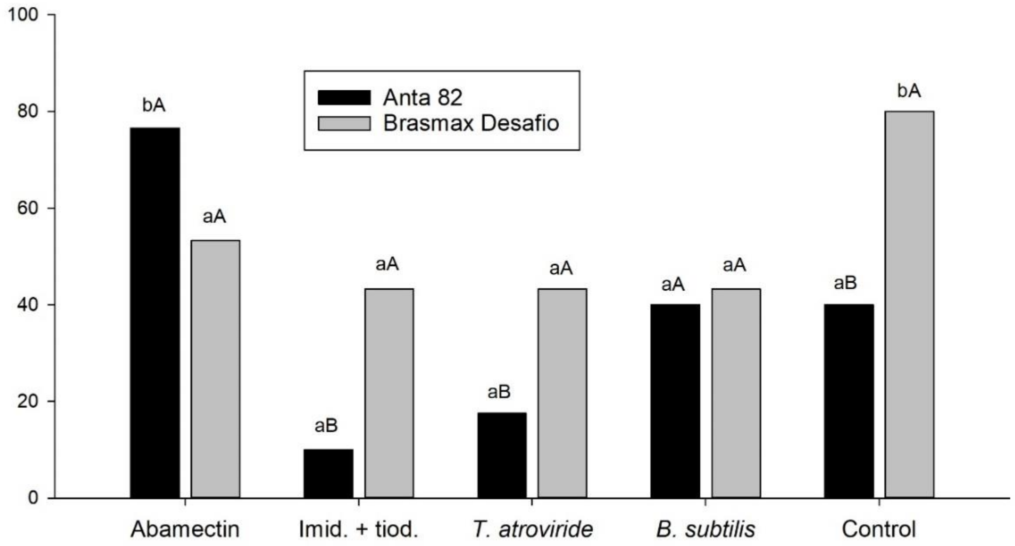
In this same way, Hussain *et al*. (2020) found that biological control mediated by *B. subtilis* is significant against *M. incongnita* for *in vitro* environment. The same was observed by Araújo; Bragante and Bragante (2012) who realized that the application of *B. subtilis* in the seeds of the susceptible cultivar 'BRS 184' decreased about 70% the presence of eggs and active forms of *Meloidogyne* spp. These positive results happen because, in association with the rhizosphere, bacteria produce toxic substances or repellents that inhibit the nematode parasitism, reducing the infestation of the root system (FREITAS, 2006).

In their research with *B. subtilis*, Araújo, Silva and Araújo (2002) proved that this bacterium directly affects nematodes orientation, by making association with the plant, in interferes in the exudates produced by the roots, that are used by the nematodes to guide themselves towards root system, and then, it affects the infection and, consequently, the reproduction of the nematode. Moreover, some *Bacillus* specie may induce resistance against nematodes (ZHOU *et al.*, 2021).

The chemical and biological controls with Imidacloprid + thiodicarb and *T. atroviride* reveal efficiency as being a strategy for the management of this species of nematode in infested areas with susceptible cultivars, which may contribute to the non-increase in nematode infestation and may contribute to decrease their populations.

The unfolding of the interaction between agents and cultivars to the number of eggs in the root (Figure 3) identified significant difference only between control agents and the control with the susceptible cultivar. On the other hand, for the resistant one, the highest number of eggs was found in treatments with Abamectin, *T. atroviride* and *B. subtilis*.

Regarding to the behavior of cultivars within each agent (Figure 3), *Trichoderma*, *B. subtilis* and Abamectin had similar responses between the cultivars, demonstrating the effect of the agents on the control of eggs for the susceptible cultivar. Similar results were obtained by Máscia (2017), but for *T. harzianum* instead. Oliveira *et al*. (2019) obtained results statistically equal also for *B. subtilis*, abamectin and *T. asperellum*, so it is possible that *Trichoderma* species have similar potential for nematode control. The effect of abamectins has already been proven in other cultures, such as for the management of *M. incognita* (MONFORT *et al*., 2006; BESSI; SUJIMOTO; INOMOTO, 2010; KUBO; MACHADO; OLIVEIRA, 2012; D'ERRICO *et al*., 2017; KHALIL; ABD EL-NABY, 2018), *Heterodera avenae* (ZHANG *et al.*, 2017) and *H. glycines* (JENSEN *et al*., 2018a; JENSEN *et al*., 2018b).

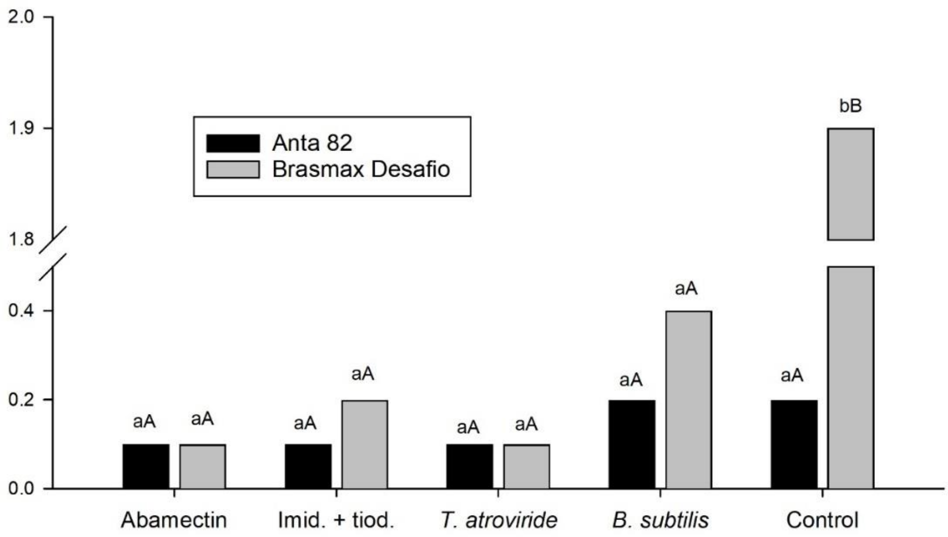


Means followed by the same letter, lowercase for cultivars and uppercase for agents, do not differ at the 5% level of significance by the Skott-Knott test.

Figure 3. Unfolding of the interaction between agents and cultivars for the number of eggs in the root (NEroot) of *P. brachyurus* in soybean plants, in function of cultivars and control agents. Alta Floresta, MT, 2018.

However, the combinations of genetic resistance of the resistant cultivar with the control via Imidacloprid + thiodicarb or *Trichoderma* were more efficient than the management only by the control agents, as visualized in the effects of the susceptible cultivar, which indicates that the strategies of chemical and biological management can be combined with the genetic resistance of plants

About the reproduction factor (RF), only considering the resistant cultivar, no differences were found between the agents of control, and also in relation to the product/control treatment (Figure 4). But, for the susceptible one, all treatments differed from the control, evidencing their effect in reducing the reproduction of *P. brachyurus*. Another aspect to emphasize about the effect of the agents in the control of reproduction is that, when any product was applied, regardless if chemical or biological, the cultivars did not differ from each other. The difference between cultivars was verified only in the controls without application of control agents.



Means followed by the same letter, lowercase for cultivars and uppercase for agents, do not differ at the 5% level of significance by the Skott-Knott test.

Figure 4: Average values of reproduction factor (RF) of soybean plants for the unfolding of the significant interaction between agents and cultivars, aiming at the control of *Pratylenchus brachyurus.* Alta Floresta - MT, 2018.

Bortolini *et al.* (2013) highlight the difficulties of the management of *P. brachyurus* in soybean fields, even in areas with crop rotation, since this nematode has polyphagous habit. The development of soybean genotypes with genetic resistance is an efficient and low-cost method to mitigate yield losses in soybean production caused by nematodes (MACHADO; ARAÚJO FILHO, 2016)

In a research carried out by Alves *et al*. (2011) in Mato Grosso state, in which 39 soybean cultivars were evaluated for resistance to *P. brachyurus*, and all cultivars were parasitized by this nematode, but in different levels of infestation. The same levels of reproduction of *P. brachyurus* were observed for the cultivars ‘M-Soy 8757’, ‘M-Soy 8850’ and ‘Aurora’, with RF = 0.88, 1.16 and 1.34, respectively. The most susceptible cultivars were ‘CD 211’ (RF=5.20), ‘Emgopa 314’ (RF=5,13) and ‘Jiripoca’ (RF=5.01). Those authors also emphasize that cultivars without any level of resistance should be avoided in areas infested by *P. brachyurus*, as they may lead to an exponential increase in populations.

In this present study, the cultivar ‘Brasmax Desafio’, which is susceptible to *P. brachyurus*, obtained low reproduction factor for this nematode when associated with any control agent, since they all differed from the control treatment which showed RF= 1.9. It means that the use of this cultivar in fields infested by *P. brachyurus* should be combined with a control agent.

CONCLUSIONS

- All the control agents are indicated to manage *Pratylenchus brachyurus* on susceptible cultivars of soybean.
- *Trichoderma atroviride* and Imidacloprid + thiodicarb are more efficient in the management of *P. brachyurus*, as they decrease the number of eggs and juveniles in roots in approximately 80%.
- The use of genetic resistance itself is efficient to manage root-lesion nematodes, and it can be combined with control agents, diversifying, then, the strategies of nematodes integrated management.
- There is no difference among the ways of application for nematode control purposes, either seed treatment, in the furrow or spraying at the V1 stage.

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