DOI: 10.37856/bja.v96i1.4244

IMPACT OF CHEMICAL COMPOUNDS INTERACTIONS ON SEED TREATMENTS TO CONTROL THE SOYBEAN NEMATODE *Pratylenchus brachyurus*

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ABSTRACT

Pratylenchus brachyurus stands out among the plant-parasitic nematodes that cause the most damage to soybean culture in Brazil. Thus, we investigated the interactions of different chemical groups in the seeds treatment for control of *P. brachyurus*. The experiments, carried out in a greenhouse, included eight treatments and six replicates in a completely randomized design. The chemical groups and interactions were: thiophanate-methyl; pyraclostrobin; fipronil; pyraclostrobin + thiophanate-methyl + fipronil and abamectin. The chemical interactions were: pyraclostrobin + thiophanate-methyl; thiophanate-methyl + fipronil; pyraclostrobin + fipronil. Seeds without product application were maintained as control. All combined products showed an antagonistic effect, however fipronil or when combined with thiophanate-methyl showed a pronounced efficacy in the control of *P. brachyurus*.

Keywords: *Glycine max*, chemical control, root lesion nematodes

IMPACTO DAS INTERAÇÕES DE COMPOSTOS QUÍMICOS NO TRATAMENTO DE SEMENTES NO CONTROLE DE *Pratylenchus brachyurus* NA CULTURA DA SOJA

RESUMO

Pratylenchus brachyurus se destaca entre as espécies de fitonematoides que mais causam prejuízos na cultura da soja no Brasil. O trabalho teve como objetivo avaliar as interações de diferentes grupos químicos no tratamento de sementes para o controle de *P. brachyurus*. Os experimentos foram conduzidos em casa de vegetação com o delineamento inteiramente casualizado, composto por oito tratamentos e seis repetições, sendo tiofanato metílico; piraclostrobina; fipronil; piraclostrobina + tiofanato metílico + fipronil e abamectina. As interações químicas foram: tiofanato metílico + piraclostrobina; tiofanato metílico + fipronil; piraclostrobina + fipronil. Tratamentos com sementes sem aplicação de produtos foram mantidos como controle. Todos os produtos combinados apresentaram efeito antagônico, porém o composto fipronil isolado ou combinado com o composto tiofanato metílico se destacou no controle de *P. brachyurus*.

Palavras-chave: *Glycine max*, controle químico, nematoide-das-lesões-radiculares

INTRODUCTION

In the last crops, nematodes have been highlighted by the elevated damages caused on soybean culture due to monoculture, deficiency in resistant varieties, inadequate management, and lack of products with greater control efficiency. Among the most important nematode groups in Brazilian Cerrado, since the 2003/2004 crop, the root lesions ones, *Pratylenchus* spp., is pointed out as the most agricultural importance (FRANCHINI et al., 2014). Many strategies for the control of these nematodes have been broadly widespread. This includes use of resistant varieties, crop rotation without hosting species and treatment of seeds (ARAÚJO et al., 2012; CORTE et al., 2014). However, the soybean resistant to this species is scarce in the market, and the variety of hosts for crop rotation have been made the *Pratylenchus brachyurus* root lesions nematode control management difficult (INOMOTO, 2007; SOUZA & INOMOTO, 2019).

Phytoparasitic nematodes may infect the root from the seedling stage, or, right at the beginning of seed germination (CAMPOS et al., 2011). Thus, the chemical control by seed treatments can reduce the damages caused by these nematodes in the initial stages of development of the plant or provide exhaust to these pathogens. Besides that, the seed treatments facilitate the production costs using extremely small amounts of the product, considering the target-organism and also reducing environmental risks (HENNING, 2005). The seed treatment is active only at the spermosphere and rhizosphere regions around the seedlings root system, so it has limited mobility, decays after a short space of time and has the absorption limited by the plant during its development (MUNKVOLD et al., 2014). Thus, the best scenario for the phytonematodes control is to work with an efficient product, reflecting the best production cost, low phytotoxicity and considering the rational and sustainable use of soil-originated resources. The main products used in the seed treatment for the phytonematodes control are abamectin, imidaclopride + thiodicarb, fluazinam + thiophanate-methyl, fluopyram and pyraclostrobin + tiophanate-methyl + fipronil. Among these products, the most used is the active ingredient abamectin, belonging to a broad Family of avermectins. The avermectins are macrocyclic lactones of 16 members produced by the soil bacteria *Streptomyces avermitilis* with insecticide, nematicide and acaricide properties (BURG et al., 1979; BULL et al., 1984). That is why the search for another active ingredients or even the combination of them aiming at multiple pathogens contributes to the expansion of new molecules in the control of phytonematodes. Besides that, changes occurred in the pathogen population due to the constant cultivation of host species associated to inadequate management can result in a lower sensitivity of the pathogen to chemical products during time, becoming necessary the search for new treatments of seed using formulations with different active ingredients (REIS et al., 2010).

In some situations, the mixture between nematicides, insecticides and fungicides may lead to the occurrence of interactions such as: (i) additive, when the product efficiency is similar or equal to the application of both individually; (ii) antagonistic, when a product interferes negatively in the efficiency of another; or (iii) synergic, when a product increases the efficiency of another by the mixture. Thus, these combinations may or may not harm control besides producing unknown effects regarding toxicology (QUEIROZ et al., 2008; PETTER et al., 2013). However, researches upon the compatibility of the mixture of different actives for the seed treatment are scarce. Some works (GARCÉS-FIALLOS & FORCELINI, 2013; ALVES & JULIATTI, 2018) have been conducted aiming at evaluating the interaction between chemical groups, however only aiming the pathogen control from the aerial part. Yet, for the seed treatment, a little is known about the interaction of the addition of fungicides and insecticides in the nematodes control. Thus, considering the nematodes importance in soybean culture and the difficulty in control them, the objective of this work was to evaluate the interactions between fungicides and insecticides in the treatment of seeds for the control of the root lesions nematode *P. brachyurus*.

MATERIAL AND METHODS

Inoculum attainment

For inoculum attainment, the soybean plant roots were carefully washed, cut into pieces of approximately 1.0 cm and milled by 5 seconds in water using a blender. The sample was processed in a centrifugal machine in accord with the Coolen & D'Herde (1972) technique. Following, using biological microscope and Peters chamber, the nematodes population was estimated and the concentration suspension (specimens. mL^{-1}) was adjusted depending on the test, for posterior inoculation.

Evaluation of *P. brachyurus* specimens penetration during time after seed treatment

Soybean seeds from the cultivar NA 7337 RR were superficially disinfested with sodium hypochlorite (1%) for 1 minute then washed in water. Right after, the treatment of seeds before plantation was conducted.

The treatments used were composed by active ingredients with the respective commercial names: Tiophanate-methyl (CERCOBIM); pyraclostrobin (COMET); fipronil (STANDAK); pyraclostrobin + tiophanate-methyl + fipronil (STANDAK TOP); and abamectin (AVICTA) 500 FS. The chemical combinations were: tiophanate-methyl + pyraclostrobin; tiophanate-methyl + fipronil; pyraclostrobin + fipronil. Besides these treatments, parcels with non-treated seeds without application of products were kept as control (Table 1).

Table 1. Products and combinations used in the experiment for control of *Pratylenchus brachyurus* in soybean culture. University of Rio Verde, Goiás State, Brazil, 2015.

TREATMENTS/ Active ingredient	**Concentration/ Formulation**	**Dose mL 100 kg semts^{-1}**	**Class**
tiophanate-methyl	500 SC	90	Fungicide
pyraclostrobin	250 SC	20	Fungicide
fipronil	250 SC	200	Insecticide
tiophanate-methyl + pyraclostrobin	SC	90 + 20	Fungicide + Fungicide
tiophanate-methyl + fipronil	SC	90 + 200	Fungicide + Insecticide
pyraclostrobin + fipronil	SC	20 + 200	Fungicide + Insecticide
pyraclostrobin + tiophanate-methyl + fipronil	200 FS	200	Fungicide, Insecticide
abamectin	500 FS	125	Nematicide, Insecticide
	-	-	

DOI: 10.37856/bja.v96i1.4244

Seeds treatment was calibrated to a quantity of 10g of seeds with a final volume solution of 50 ml. After the treatment of seeds with the respective products and possible combinations (Table 1), two seeds were put in each recipient (plastic cups) with volume of 100mL of substrate (dampened thick sand) and kept in a temperature of 24 ± 3º C with soil humidity between 65 – 75%, under greenhouse conditions. After 48h from sowing (time enough for root emission), the sand of each cup was infested with 500 specimens of *P. brachyurus*, following the methodology proposed by Campos et al. (2011). After 5, 10 and 15 days after inoculation, the seedlings and plants were carefully removed from the recipients, pouring water in their edges.

The root systems washed were submitted to tissue clarification in sodium hypochlorite at 1,5% for 6 minutes. Next, they were transferred to glass tubes for coloring with acid fuchsine (adapted from Byrd et al., 1983). Then, a glycerin solution was applied to all roots and the roots were put in glass slide for observation in the inverted microscope. Thus, the number of *P. brachyurus* specimens penetrated in the root system during time was quantified. In this test, it was used a completely random design, with nine treatments in six repetitions x 3 evaluation times after inoculation.

Evaluation of *P. brachyurus* specimens population after treatment of seeds in greenhouse after 30 and 60 days of sowing

Soybean seeds from cultivar NA 7337 RR were disinfested as previously described then the treatment of seeds before plantation was conducted. After treatment of seeds with respective products and possible combinations (Table 1), two seeds were sowed in each 8L-vase containing a mix of sand and enhanced substrate (2:1) and kept in greenhouse in a temperature of 24 ± 3º C and soil humidity between 65-75%, under greenhouse condition. After 48h of sowing, about 1000 *P. brachyurus* specimens in 5 mL water suspension were put next to the soybean seeds.

Therefore, 30 and 60 days after sowing, plants with the entire root system were carefully removed and conditioned in plastic bags together with the soil obtained from rizosphere, identified and taken to the Laboratory of Nematology of the University of Rio Verde, Goiás state, Brazil. Then, the root systems were carefully washed with current water. Following, roots were kept on filter paper until the elimination of water excess and then the roots and aerial parts fresh weight were obtained. Next, roots were cut into pieces of approximately 1cm and milled for 10 seconds in water using a blender, in accord with method by Coolen & D'Herde (1972).

For the attainment of soil population, and number of specimens per 100 cm^3 of soil, there was used the method of fluctuation and centrifugation, as described by Jenkins (1964). The *P. brachyurus* quantification, in each suspension, was conducted using a *Peters* chamber and a biological microscope. After data attainment, there were determined the total number of *P. brachyurus* per root system, number of *P. brachyurus* / root grass and soil, and penetration percentage. The reproduction factor (Fr) for *P. brachyurus* was calculated by the final nematodes population (Pf) divided by the nematodes population initially inoculated (Pi). In this experiment, a completely randomized design was used, with nine treatments in six repetitions.

Statistical Analysis

All tests were repeated for data confirmation, using a completely randomized design. Data were submitted to Shapiro-Wilk and Bartlett ($p > 0,05$) tests in order to confirm the normal distribution and homoscedasticity, respectively, to variance analysis (ANOVA). Otherwise, the data represented by the number of nematodes were transformed in root of x + 0.5 for ANOVA conduction. Adopting these parameters, data were submitted to one-way ANOVA and the treatments means were compared by Scott-Knott (1974) test ($p < 0,05$), with analyzes conducted using the statistical software SISVAR. There were also conducted analyzes of Colby (COLBY 1967) in order to check the interactions between the compounds. Interaction relation: Obs (observed results) / Esp (expected results) > 1 (synergic effect), Obs/Esp = 1 (additive effect), Obs/Esp < 1 (antagonistic effect).

RESULTS AND DISCUSSION

Evaluation of *P. brachyurus* specimens penetrations during time after treatment of seeds

For root weight after 5 days after inoculation, it was verified that treatment 4, which contained tiophanate-methyl + pyraclostrobin at doses of 90 + 30 mL ha^{-1} and the treatment that contained abamectin at dose of 125 mL ha^{-1} did not statistically differ from control, presenting the greater root weights (Table 1), followed by treatment 7 containing pyraclostrobin + tiophanate-methyl + fipronil at dose of 200 mL ha^{-1} (Table 1).

For root weight at 10 days after inoculations, it was verified that treatment D containing abamectin provided the highest root weights, statistically different from control and other treatments. It is followed by treatment 4 containing tiophanate-methyl + pyraclostrobin, treatment 7 containing pyraclostrobin + tiophanate-methyl + fipronil and control, which also presented greater root weights, statistically differing from other treatments (Table 2). For aerial part weight at 10 days after inoculation, it was verified that all treatments containing products presented better improvements in aerial part weights, statistically differing from control (Table 2).

For root weights at 15 days after inoculation, it was verified that similar to 5 days after inoculation, treatment 4 containing tiophanate-methyl + pyraclostrobin and treatment 8 containing abamectin did not statistically differ from control, presenting greater roots weights. It was followed by treatment 7 containing pyraclostrobin + tiophanate-methyl + fipronil (Table 2). For aerial part weights at 15 days after inoculation, it was verified that treatment 2 containing pyraclostrobin at dose of 20 mL ha^{-1}, treatment 4 containing tiophanate-methyl + pyraclostrobin, treatment 7 containing pyraclostrobin + tiophanate-methyl + fipronil and treatment 8 containing abamectin did not statistically differ from control, presenting the highest aerial part weights (Table 2). In general, the combinations of pyraclostrobin + tiophanate-methyl and the abamectin isolated compound presented higher improvements in the growth of plants (root and aerial part) in relation to other combinations.

For evaluation of *P. brachyurus* specimens total number in roots by penetration test at 5 days after inoculation, it was verified that treatment 1 containing tiophanate-methyl at dose of 90 mL ha^{-1}, treatment 2 containing pyraclostrobin at dose of 20 mL ha^{-1}, treatment 3 containing fipronil at dose of 200 mL ha^{-1}, treatment 6 containing pyraclostrobin + fipronil at dose of 20 + 200 mL ha^{-1}, and treatment 8 containing abamectin presented lower nematode penetrations in roots, statistically differing from control and other treatments (Figure 1).

For evaluation of penetration at 10 days after inoculation, it was observed that treatment 1 containing tiophanate-methyl, treatment 2 containing pyraclostrobin and treatment 6 containing pyraclostrobin + fipronil presented lower nematodes penetrations in roots, differing from control and other treatments (Figure 1). However, at 15 days after inoculation it was verified that treatment 1 containing tiophanate-methyl, treatment 2 containing pyraclostrobin, treatment 3 containing fipronil, treatment 5 containing tiophanate-methyl + fipronil at doses of 90 + 200 mL ha^{-1}, treatment 6 containing pyraclostrobin + fipronil, and treatment 8 containing abamectin presented lower nematode penetration in roots, differing from control and other treatments (Figure 1).

Table 2. Root weight (g) at 5, 10 and 15 days after inoculation in function of treatment of seeds by different interactions of chemical groups. University of Rio Verde, Goiás State, Brazil, 2015.

TREATMENTS	Dose mL ha^{-1} ou 100 kg $semts^{-1}$	Root weight (g)
tiophanate-methyl	90	0.27 c
pyraclostrobin	20	0.31 c
fipronil	200	0.25 c
tiophanate-methyl + pyraclostrobin	90 + 20	1.16 a
tiophanate-methyl + fipronil	90 + 200	0.21 c
pyraclostrobin + fipronil	20 + 200	0.35 c
pyraclostrobin + tiophanate-methyl + fipronil	200	0.69 b
abamectin	125	1.17 a
control	-	1.00 a
CV(%)		**44.90**

--10 days after inoculation------------------------------------

TREATMENTS	Root weight (g)	Aerial part weight (g)
tiophanate-methyl	0.28 c	1.34 a
pyraclostrobin	0.44 c	1.46 a
fipronil	0.46 c	1.47 a
tiophanate-methyl + pyraclostrobin	1.33 b	1.59 a
tiophanate-methyl + fipronil	0.91c	1.42 a
pyraclostrobin + fipronil	0.82 c	1.48 a
pyraclostrobin + tiophanate-methyl + fipronil	1.58 b	1.66 a
abamectin	2.68 a	1.49 a
control	1.67 b	1.07 b
CV (%)	**39.46**	**12.31**

--15 days after inoculation------------------------------------

TREATMENTS	Root weight (g)	Aerial Part Weight (g)
tiophanate-methyl	0.41 c	1.44 b
pyraclostrobin	0.63 c	1.54 a
fipronil	0.51 c	1.42 b
tiophanate-methyl + pyraclostrobin	2.19 a	1.74 a
tiophanate-methyl + fipronil	0.60c	1.22 b
pyraclostrobin + fipronil	0.64 c	1.46 b
pyraclostrobin + tiophanate-methyl + fipronil	1.73 b	1.55 a
abamectin	2.60 a	1.61 a
control	2.33 a	1.63a
CV (%)	**36.43**	**13.49**

Means followed by the same letter in each column did not statistically differ between each other by Scott-Knott test at 5% of probability.

In a general way, treatment 1 containing tiophanate-methyl, treatment 2 containing pyraclostrobin and treatment 6 containing pyraclostrobin + fipronil presented significant lower *P. brachyurus* penetrations in roots in all times evaluated. At 15 days after evaluation, all treatments presented lower number of nematodes in roots in relation to control, except treatments 4 containing tiophanate-methyl + pyraclostrobin and 7 containing pyraclostrobin + tiophanate-methyl + fipronil (Figure 1). The increase of nematode penetration at 10 days after inoculation for most of the treatments may be related to the increase of root system and consequently higher availability of roots (penetrations points) for the parasite. Yet, at 15 days after inoculation the population presented itself lower, probably in function of the lower root system development and lower quantity of penetrations points when compared to the observed at 10 days after inoculation.

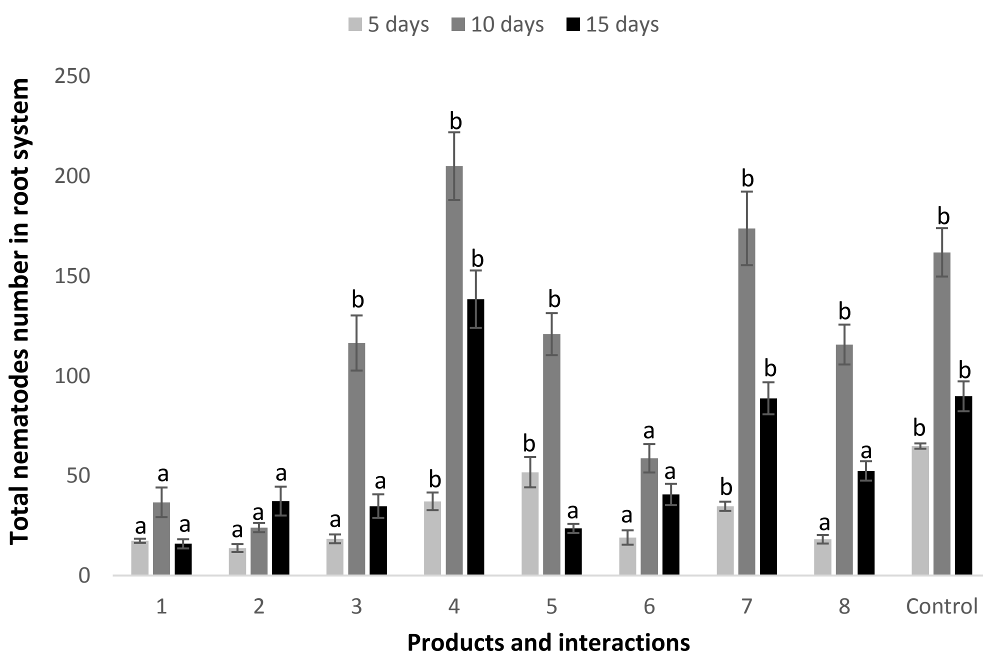

Figure 1. Evaluation of *P. brachyurus* specimens penetrations during time (5, 10 and 15 days) after treatment of seeds by different interactions of chemical groups. Means followed by the same letter in time did not statistically differ according with Scott-Knott test at 5% of probability. Bars indicate means standard errors (E±ME). Treatments: **1** = tiophanate-methyl (Cercobim); **2** = pyraclostrobin (Comet); **3** = fipronil (Standak); **4** = tiophanate-methyl + pyraclostrobin; **5** = tiophanate-methyl + fipronil; **6** = pyraclostrobin + fipronil; **7** = pyraclostrobin + tiophanate-methyl + fipronil (Standak Top); **8** = abamectin (Avicta). University of Rio Verde, Goiás State, Brazil, 2015.

Evaluation of *P. brachyurus* specimens populations after treatment of seeds in greenhouse at 30 and 60 days after sowing

For total number of nematodes per root system at 30 days, it was verified that treatments containing fipronil and treatment containing abamectin presented lower nematodes population at 30 days after sowing, statistically differing from control and other treatments (Table 2). However, the only treatment that did no differ in relation to control was treatment 1 containing tiophanate-methyl, presenting higher nematode population in relation to the others (Table 2). For total number of nematodes on soil, no statistical differences occurred between treatments and control. At 60 days, it was verified that treatment 1 containing tiophanate-methyl at dose of 90 mL/100kg of seeds, treatment 2 containing pyraclostrobin at dose of 20 mL/100kg of seeds and treatment 8 containing abamectin at dose of 125 mL/100kg of seeds presented the lowest nematodes populations, however they did not statistically differ from control (Table 2). For number of nematodes on soil, there was no statistical differences of populations between treatments in relation to control.

For total number of nematodes (total soil quantity + roots in the vase interior) at 30 days, it was verified that treatment 8 containing abamectin presented lower nematodes population in relation to control and other treatments. Followed by treatment 3 containing fipronil and by treatment 5 containing tiophanate-methyl + fipronil (Table 3). For treatments with presence of products, the reproduction factor varied from 0.28 (Treatment 8 containing abamectin at dose of 125 mL/100 kg of seeds) to 1.95 (Treatment 2 containing pyraclostrobin at dose of 20 mL/100kg of seeds). The lowest reproduction factors were presented by treatments 8, 3 and 5 (Table 3). Consequently, lower penetration percentages were verified by treatment 8 containing abamectin (10.31%), by treatment 3 containing fipronil (23.28%) and by treatment 5 containing tiophanate-methyl + fipronil (42.47%). Yet, other treatments presented penetration percentages above 50% (Table 3). At 60 days after sowing, all treatments presented penetration percentage above 70%. It is important to point out that in this experiment we did not prioritize productivity enhancement once these tests were not conducted at field. Thus, at 60 days was not possible to evaluate the differences regarding the future plant productive development.

Table 2. *Pratylenchus brachyurus* reproduction (total number of nematodes per root system) in soybean plants at 30 and 60 days after using treatment of seeds. University of Rio Verde, Goiás State, Brazil, 2015.

TREATMENTS	Dose mL ha^{-1} ou 100 kg seeds^{-1}	Total number of nematodes/root system	Number of nematodes on soil
tiophanate-methyl	90	666.50 c	24.47 ns
pyraclostrobin	20	493.50 b	48.52
fipronil	200	213.00 a	25.40
tiophanate-methyl + pyraclostrobin	90 + 20	465.83 b	27.18
tiophanate-methyl + fipronil	90 + 200	388.50 b	13.40
pyraclostrobin + fipronil	20 + 200	451.33 b	22.83
pyraclostrobin + tiophanate-methyl + fipronil	200	512.17 b	25.93
abamectin	125	94.33 a	6.20
control	-	914.83 c	30.93
CV (%)		**59.83**	**41.81***

---60 days after inoculation---------------------------------

TREATMENTS	Dose mL ha^{-1} ou 100 kg seeds^{-1}	Total number of nematodes/ root system	Number of nematodes on soil
tiophanate-methyl	90	999.0 a	12.63 ns
pyraclostrobin	20	977.8 a	10.95
fipronil	200	1481.7 b	13.33
tiophanate-methyl + pyraclostrobin	90 + 20	1971.5 b	26,25
tiophanate-methyl + fipronil	90 + 200	1857.7 b	11.98
pyraclostrobin + fipronil	20 + 200	1754.2 b	8.65
pyraclostrobin + tiophanate-methyl+ fipronil	200	1886.5 b	9.27
abamectin	125	1154.0 a	17.72
control	-	1282.7 a	15.68
CV (%)		**22.28***	**39.47***

Means followed by the same letter in each column did not statistically differ between each other by Scott-Knott test at 5% of probability. * Data transformed into SQRT (x + 0.5). Treatments: Treat. 1 = **A** = tiophanate-methyl (Cercobim); Treat. 2 = **B** = pyraclostrobin (Comet); Treat. 3 = **C** = fipronil (Standak); Treat. 4 = **A+B** = tiophanate-methyl + pyraclostrobin; Treat. 5 = **A+C** = tiophanate-methyl + fipronil; Treat. 6 = **B+C** = pyraclostrobin + fipronil; Treat. 7 = **A+B+C** = pyraclostrobin + tiophanate-methyl + fipronil (Standak Top); Treat. 8 = **D** = abamectin (Avicta); Treat. 9 = Control. ns = not significant

Table 3. Total number of nematodes, reproduction and penetration (%) factors, based on the number of *Pratylenchus brachyurus* per root gram at 30 days of sowing, using treated seeds and under greenhouse conditions. University of Rio Verde, Goiás State, Brazil, 2015.

TREATMENTS	Dose mL ha^{-1} or 100 kg seeds^{-1}	Total nematodes	Reproduction factor	(%) Penetration
tiophanate-methyl	90	2801.00 c	1.40	72.85
pyraclostrobin	20	3898.00 c	1.95	53.94
fipronil	200	1950.00 b	0.98	23.28
tiophanate-methyl + pyraclostrobin	90 + 20	2562.67c	1.28	50.92
tiophanate-methyl + fipronil	90 + 200	1581.00 b	0.79	42.47
piraclostrobina + fipronil	20 + 200	2272.67 c	1.14	49.34
piraclostrobina + tiophanate-methyl + fipronil	200	2580.33 c	1.29	55.98
abamectin	125	560.67 a	0.28	10.31
control	-	3685.67 c	1.84	100.00
CV (%)		**28.23***		
----60 days after inoculation----				
TREATMENTS	**Dose mL ha^{-1} or 100 kg semts^{-1}**	**Total nematodes**	**Reproduction Factor**	**(%) Penetration**
tiophanate-methyl	90	2756.00 ns	1.37	77.88
pyraclostrobin	20	2612.67	1.30	76.23
fipronil	200	3763.33	1.88	100.00
tiophanate-methyl + pyraclostrobin	90 + 20	5518.00	2.75	100.00
tiophanate-methyl + fipronil	90 + 200	4434.33	2.21	100.00
pyraclostrobin + fipronil	20 + 200	4027.33	2.01	100.00
pyraclostrobin + tiophanate-methyl + fipronil	200	4329.00	2.16	100.00
abamectin	125	3371.00	1.68	89.97
control	-	3506.33	1.75	100.00
CV (%)		**20.10**		

Means followed by the same letter in each column did not statistically differ between each other by Scott-Knott test at 5% of probability. * Data transformed into SQRT (x + 0.5). Treatments: Treat. 1 = **A** = tiophanate-methyl (Cercobim); Treat. 2 = **B** = pyraclostrobin (Comet); Treat. 3 = **C** = fipronil (Standak); Treat. 4 = **A+B** = tiophanate-methyl + pyraclostrobin; Treat. 5 = **A+C** = tiophanate-methyl + fipronil; Treat. 6 = **B+C** = pyraclostrobin + fipronil; Treat. 7 = **A+B+C** = pyraclostrobin + tiophanate-methyl + fipronil (Standak Top), treat. 8 = **D** = abamectin (Avicta), treat. 9 = control.

ns = not significant

DOI: 10.37856/bja.v96i1.4244

All interactions presented antagonistic behavior by Colby analysis (Table 4), indicating that products interaction was not efficient in controlling the nematode. The formulated product containing pyraclostrobin + tiophanate-methyl + fipronil (Treatment 7) and the combination of the other products presented low nematodes control and high severity of disease in the plants rizosphere (Table 4). However, it is important to highlight that the compound fipronil, isolated or combined with the compound tiophanate-methyl stood out in the control of *P. brachyurus* in relation to other treatments.

Table 4. Colby analysis for the interaction between fungicides and insecticides at 30 days of sowing. University of Rio Verde, Goiás State, Brazil, 2015.

[1]TREATMENTS	Dose mL 100 kg seeds^{-1}	Severity (%)	Observed Control (%)	Expected Control (%)	[2]Interaction relation
tiophanate-methyl + pyraclostrobin	90 + 20	50.92	49.08	71.48	0.68
tiophanate-methyl + fipronil	90 + 200	42.47	57.53	76.22	0.75
pyraclostrobin + fipronil	20 + 200	49.34	50.66	72.37	0.69
pyraclostrobin + tiophanate-methyl + fipronil	200	55.99	44.01	51.78	0.84

[1]Treat. 4 = **A+B** = tiophanate-methyl + pyraclostrobin; Treat. 5 = **A+C** = tiophanate-methyl + fipronil; Treat. 6 = **B+C** = pyraclostrobin + fipronil; Treat. 7 = **A+B+C** = pyraclostrobin + tiophanate-methyl + fipronil (Standak Top); Treat. 8 = **D** = abamectin (Avicta); Treat. 9 = Control. [2]Interaction relation Obs/Esp > 1 (synergic) Obs/Esp = 1 (additive) Obs/Esp < 1 (antagonistic)

The higher penetration percentage at 10 days of sowing in the first test may be related to the higher radicles availability in this period. However, from 15 days after sowing the penetration reduction occurred again, except for treatments 4 and 7. Interestingly, the isolated compounds pointed out in most of the evaluation intervals. Possibly, the products which pointed out in control had more contact with tegument, suggesting a greater residual effect.

Faske & Starr (2007) observed that the higher mortality of *M. incognita* and *R. reniformis* in cotton culture was associated to the higher abamectin concentration in the seed tegument when compared to radicles. However, in the second test some products such as tiophanate-methyl and pyraclostrobin did not show present to be positive in the nematode control like in the first test. Such results suggest that the performance of these products may have been affected by different abiotic conditions, compromising nematodes control. Yet, authors Ribeiro et al. (2014) reported that the compounds of pyraclostrobin + tiophanate-methyl + fipronil reduced *P. brachyurus* populations in soybean plants under hydric stress conditions in greenhouse.

Besides that, in the first test at 15 days after inoculation it was verified greater aerial part weights in all treatments with pyraclostrobin. The combination of pyraclostrobin with other compounds or even isolated have been presenting positive effect on plants growth (SWOBODA & PEDERSON, 2009; AMARO et al., 2018). Treatment of seed is one of the main practices in the management of plants diseases. However, it is important to highlight that control methods complement each other and management is fundamental in order to assure high productivities (MUNKVOLD et al., 2014). Thus, our results demonstrate that the product efficiency depends on many factor and other application technologies may also complement the seeds treatment, such as the application of the product in the plantation furrow (CORTE et al., 2014).

Gourd et al. (1993) reported that the activity of chemical products on soil causes the direct death of *Meloidogyne* juveniles, inhibits hatching, reduces mobility of J2's, and provokes disorientation in penetration. However, by the time these concentrations are reduced by degradation or leaching, these effects become reversible (SILVA et al., 2019). In fact, the greater residual power to tegument determines a lower nematodes penetration in roots. It is possible to observe that in the first test the combination of pyraclostrobin + tiophanate-methyl allowed a greater penetrations of nematodes in roots, being poorly efficient in controlling penetration in relation to others treatments. However, the combination of pyraclostrobin + fipronil presented lower nematodes penetration in all evaluations conducted. Generally, our results showed that the combination of tiophanate-methyl + fipronil had better control both in the first and in the second test, in relation to other combinations.

Unlike fipronil, that is known as an insecticide, the other compounds tiophanate-methyl and pyraclostrobin are fungicides. Fipronil is an insecticide from the chemical group pyrazole, which acts by contact and ingestion, where the nervous system is interrupted, blocking the chlorine gates mediated by GABA-pyrazole (SCHARF & SIEGFRIED, 1999; CUI et al., 2017). This indicates that the action mode of these molecules in the phytopathogen control are different, with the insecticide fipronil being, in that case, more deleterious to nematode than other products. Therefore, our results demonstrate that fipronil, combined or not, had effect in the control of nematodes equivalent to the standard product abamectin. However, a little is known about the combined action of these fungicides, insecticides and nematicides in nematodes control.

At 60 days after sowing in the greenhouse test, the reproduction factors presented themselves as being more elevated when compared to evaluations conducted at 30 days after sowing. Higher reproduction factors values are reached from 60, 75 and 90 days after inoculation (INOMOTO, 2011), depending on the used cultivar. Higher root availability and occurrence of a new nematode cycle suggest FR values above 1.0 in the test studied. Such fact can be explained by a quick response of the products in the first stages of the plant until 30 days, allowing the changes in nematodes population to be measured yet in its first cycle. It is important to highlight that in this experiment we did not prioritize productivity increase, once these tests were not conducted in field. Thus, after 60 days of inoculation the differences between products could be highlighted regarding increase in production of plants.

The antagonistic effect of the combination of active ingredients in *P. brachyurus* control can be explained by the laboratorial manipulation of the compounds without taking in account the addition of excipient compounds that aid in the stability of the formulation. In fact, the presence of these excipient compounds is primary for the development of a product, because although they do not provide direct activities in formulation, their presence in the formula influences the liberation system and the bioavailability of the active principle (FERREIRA et al., 2013). That is why in-depth studies upon the interaction of molecules for nematodes control are clearly relevant, once the behavior of these compounds can be instable and the nematodes population susceptible to constant genetic changes. However, other field investigations or *in vitro* tests are necessary in order to clarify these questions.

CONCLUSION

The seed treatment for the phytonematodes control has been a great challenge for agriculture, but few studies have been considered the combined action of chemical molecules in the control of this phytoparasite. In our work, we presented how different combinations of fungicides and insecticides behave in *P. brachyurus* management in greenhouse soybean culture. Although our results having demonstrated that the combined products presented an antagonistic effect, it is important to point out that these results may vary in function of the interaction of these molecules, doses and environmental effect. Thus, another investigation with these products are necessary in order to clarify the behavior of these interactions under effect of many factors. Indeed, the lower nematode penetration at the initial stage allows a better establishing of roots on the soil and seedling development. In this context, assuring a protection of seed in the first days of radicle development is crucial, once after germination the rhizosphere contacts the soil, whether by biotic aspect with beneficial microorganisms or abiotic aspects. That is the reason why we must understand the agrotoxin application technologies such as treatment of seeds in order to prevent the early nematodes attack contributes to greater plant defense and high productivity in the future. The results strengthened the value of seeds treatment and the combination of active ingredients such as fipronil and tiophanate-methyl, which aims multiple pathogens. However, better comprehension about the chemical group's interaction for the control of plant parasite nematodes requires field investigation.

REFERENCES

ALVES, V. M.; JULIATTI, F. C. 2018. Fungicidas no manejo da ferrugem da soja, processos fisiológicos e produtividade da cultura. **Summa Phytopathology**, Botucatu, v.44, n.3, p.245-251. Available at: <https://www.scielo.br/pdf/sp/v44n3/0100-5405-sp-44-3-0245.pdf> Accessed on: Nov. 13, 2019.

AMARO, A. C. E.; RAMOS, A. R. P.; MACEDO, A. C.; ONO, E. O.; RODRIGUES, J. D. 2018. Effects of the fungicides azoxystrobin, pyraclostrobin and boscalid on the physiology o Japanese cucumber. **Scientia Horticulturae**, v.228, p.66-75. Available at: <https://doi.org/10.1016/j.scienta.2017.10.016> Accessed on: Oct. 1, 2020.

ARAÚJO, F. F.; BRAGANTE, R. J.; BRAGRANTE, C. E. 2012. Controle genético, químico e biológico de meloidoginose na cultura da soja. **Pesquisa Agropecuária Tropical**, Goiânia, v.42, n.2, p.220-224. Available at: <http://dx.doi.org/10.1590/S1983-40632012000200013> Accessed on: Mar. 21, 2015.

BULL, D. L.; IVIE, G. W.; MACCONNELL, J. G.; GRUBER, V. F.; KU, C. C.; ARISON, B. H.; STEVENSON, J. M.; VANDENHEUVEL, W. J. A. 1984. Fate of avermectin B1a in soil and plants. **Journal of Agricultural and Food Chemistry**, v.32, p.94-102. Available at: <https://doi.org/10.1021/jf00121a025> Accessed on: Nov. 13, 2019.

BURG, R. W.; MILLER, B. M.; BAKER, E. E.; BRINBAUM, J.; CURRIE, S. A.; HARTMAN, R.; KONG, Y.; MONAGHAN, R. L.; OLSON, G.; PUTTER, I.; TUNAC, J. B.; WALLICK, H.; STAPLEY, E. O.; OIWA, R.; OMURA, S. 1979. Avermectins, new family of potent anthelmintic agents: producing organism and fermentation. **Antimicrobial Agents and Chemotherapy**, v.15, n.3, p.361-367. Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC352666/> Accessed on: Nov. 13, 2019.

BYRD, Jr. D. W.; KIRKPATRICK, T.; BARKER, K. R. 1983. An improved technique for clearing and staining plant tissue for detection of nematodes. **Journal of Nematology**, v.14, n.1, p.142-143. Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2618249/pdf/142.pdf> Accessed on: Nov. 8, 2014.

CAMPOS, H. D.; CAMPOS, V. P.; SILVA, C. R. J.; SILVA, P. C. H. L.; COSTA, L. S. A. S.; TERRA, W. C. 2011. Atração e penetração de *Meloidogyne javanica* e *Heterodera glycines* em raízes excisadas de soja. **Ciência Rural**, Santa Maria, v.41, n.9, p.1496-1502. Available at: <https://www.scielo.br/pdf/cr/v41n9/a11211cr3264.pdf> Accessed on: Mar. 16, 2015.

COLBY, S. R. 1967. Calculating Synergistic and Antagonistic Responses of Herbicide Combinations. **Weed Science Society of America**, Cambridge University Press, v.15, n.1, p.20-22. Available at: <https://doi.org/10.2307/4041058> Accessed on: May. 24, 2020.

COOLEN, W. A.; D'HERDE, D. J. 1972. A method for the quantitative extraction of nematodes from plant tissue. **Ghent**, State Agricultural Research Centre, Belgium, 77pp.

CORTE, G. D.; PINTO, F. F.; STEFANELLO, M. T.; GULART, C.; RAMOS, J. P.; BALARDIN, R. S. 2014. Tecnologia de aplicação de agrotóxicos no controle de fitonematoides em soja. **Ciência Rural**, Santa Maria, v.44, n.9, p.1534-1540. Available at: <https://www.scielo.br/pdf/cr/v44n9/0103-8478-cr-44-09-01534.pdf> Accessed on: Apr. 29, 2020.

CUI, J. K.; HUANG, W. K.; PENG, H. 2017. Efficacy evaluation pf seed-coating compounds against cereal cyst nematode and root lesion nematodes on wheat. **Plant Disease**, v.101, n.3, p.428-433. Available at: <https://doi.org/10.1094/PDIS-06-16-0862-RE> Accessed on: Apr. 29, 2020.

FASKE, T. R.; STARR, J. L. 2007. Cotton root protection from plant-parasitic nematodes by abamectin-treated seed. **Journal of Nematology**, Hanover, v.39, n.1, p.27-30. Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2586483/> Accessed on: Nov. 13, 2015.

FERREIRA, T. F.; MOURÃO, A. S. R.; RIBEIRO, L. A. L.; FREITAS, M. B. 2013. Estudo comparativo da influência dos excipientes na qualidade de hidroclorotiazida 25 mg em medicamentos referência e genéricos. **Revista de Ciências Farmacêuticas Básica e Aplicada**, v.34, n.1, p.63-68. Available at: <https://rcfba.fcfar.unesp.br/index.php/ojs/article/view/238> Accessed on: Oct. 1, 2020.

FRANCHINI, J. C.; DEBIASI, H.; DIAS, W. P.; RAMOS JUNIOR, E. U.; SILVA, J. F. V. 2014. Perda de produtividade da soja em área infestada por nematoide das lesões radiculares na região médio norte do Mato Grosso. In: BERNARDI, A. C. de C.; NAIME, J. de M.; RESENDE, A. V. de; BASSOI, L. H.; INAMASU, R. Y. (Ed.). **Agricultura de precisão: resultados de um novo olhar**, Brasília: Embrapa, p.274-278. Available at: <https://www.embrapa.br/busca-de-publicacoes/-/publicacao/1002047/> Accessed on: Apr. 11, 2020.

GARCÉS-FIALLOS, F. R.; FORCELINI, C. A. 2013. Controle comparativo da ferrugem asiática da soja com fungicida triazol ou mistura de triazol + estrobilurina. **Bioscience Journal,** Uberlândia, v.29, n.4, p.805-815. Available at: <http://www.seer.ufu.br/index.php/biosciencejournal/article/view/15029> Accessed on: Nov. 25, 2015.

GOURD, T. R.; SCHMITT, D. P.; BARKER, K. R. 1993. Use of nematodes as biomonitors of nonfumigant nematicide movement through field soil. **Journal of Nematology**, Hanover, v. 25, n.1, p. 63-70. Available at: <https://pubmed.ncbi.nlm.nih.gov/19279744/> Accessed on: Nov. 8, 2014.

HENNING, A. A. 2005. Patologia e Tratamento de Semente: Noções Gerais. **Embrapa Soja Documentos (INFOTECA-E)**, Londrina, 52p. Available at: <https://www.infoteca.cnptia.embrapa.br/infoteca/bitstream/doc/469530/1/documento264.pdf> Accessed on: Oct. 20, 2015.

INOMOTO, M. M.; MACHADO, A. C. Z.; ANTEDOMENICO, S. R. 2007. Reação de *Brachiaria* spp. e *Panicum maximum* a *Pratylenchus brachyurus*. **Fitopatologia Brasileira**, v.32, p.341-344. Available at: <http://dx.doi.org/10.1590/S0100-41582007000400009> Accessed on: Nov. 27, 2019.

INOMOTO, M. M. 2011. Avaliação da resistência de 12 híbridos de milho a *Pratylenchus brachyurus*. **Tropical Plant Pathology**, vol.36, n.5, p.308-312. Available at: <http://dx.doi.org/10.1590/S1982-56762011000500006> Accessed on: Nov. 27, 2019.

JENKINS, W. R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. **Plant Disease Reporter**, v.48, n.9, p.692. Available at: <https://www.cabdirect.org/cabdirect/abstract/19650801105> Accessed on: Nov. 10, 2014.

MUNKVOLD, G. P.; WATRIN, C.; SCHELLER, M.; ZEUN, R.; OLAYA, G. 2014. Benefits of chemical seed treatments on crop yield and quality. Global perspectives on the health of seeds and plant propagation material. **Plant Pathology in the 21st Century**, p.89-103. Available at: <https://link.springer.com/book/10.1007%2F978-94-017-9389-6> Accessed on: Nov. 10, 2019.

PETTER, F. A.; SEGATE, D.; ALMEIDA, F. A.; NETO, F. A.; PACHECO, L. P. 2013. Incompatibilidade física de misturas entre inseticidas e fungicidas. **Comunicata Scientiae**, Piauí, v.4, n.2, p.129-138. Available at: <https://www.academia.edu/27798603/Incompatibilidade_f%C3%ADsica_de_misturas_entre_inseticidas_e_fungicidas> Accessed on: Jul. 11, 2019.

QUEIROZ, A. A.; MARTINS, J. A. S.; CUNHA, J. P. A. R. 2008. Adjuvantes e qualidade da água na aplicação de agrotóxicos. **Bioscience Journal**, Uberlândia, v.24, n.4, p.8-19. Available at: <http://www.seer.ufu.br/index.php/biosciencejournal/article/view/6923> Accessed on: Nov. 10, 2019.

REIS, E. M.; REIS A. C.; FORCELINI, C. A. 2010. **Manual de fungicidas: guia para o controle químico de doenças de plantas**, Passo Fundo, 226p.

RIBEIRO, L. M.; CAMPOS, H. D.; DIAS-ARIEIRA, C. R.; NEVES, D. L.; RIBEIRO, G. C. 2014. Effect of soybean seed treatment on the population dynamics of *Pratylenchus brachyurus* under water stress conditions. **Bioscience Journal**, Uberlândia, v.30, n.3, p.616-622. Available at: <http://www.seer.ufu.br/index.php/biosciencejournal/article/view/18054> Accessed on: Sep. 30, 2020.

SILVA, E. H.; CAMPOS, V. P.; ROCHA, F. S.; ZEVIANI, W. M.; TERRA, W. C.; RIGITANO, R. L. O. 2019. Efeito do aldicarbe no ciclo de vida de *Meloidogyne incognita*. **Revista de Agricultura Neotropical**, Cassilândia, v.6, n.3, p.82-91. Available at: <https://periodicosonline.uems.br/index.php/agrineo/article/view/2164/2992> Accessed on: Oct. 1, 2020.

SCHARF, M. E.; SIEGFRIED, B. D. 1999. Toxicity and neurophysiological effects of fipronil and fipronil sulfone on the western corn rootworm (Coleoptera: Chrysomelidae). **Insect Biochemistry and Physiology**, v.40, p.150-156. Available at: <https://doi.org/10.1002/(SICI)1520-6327(1999)40:3<150:AID-ARCH4>3.0.CO;2-5> Accessed on: Oct. 21, 2015.

SCOTT A. J.; KNOTT M. 1974. A cluster analysis method for grouping means in the analysis of variance. **Biometrics**, v.30, p.507-512. Available at: <https://www.jstor.org/stable/2529204> Accessed on: Apr. 21, 2020.

SOUZA, V. H. M.; INOMOTO, M. M. 2019. Host suitability of grain sorghum and sudangrass for *Pratylenchus Brachyurus*. **Scientific Communication Plant Parasitology**, São Paulo, Arq.

DOI: 10.37856/bja.v96i1.4244

Inst. Biol. v.86, p.1-4. Available at: <https://www.scielo.br/pdf/aib/v86/1808-1657-aib-86-e0262019.pdf> Accessed on: Apr. 21, 2020.

SWOBODA, C.; PEDERSEN, P. 2009. Effect of Fungicide on Soybean Growth and Yield. **Agronomy Journal**, v.101, n.2, p.352-356. Available at: <https://doi.org/10.2134/agronj2008.0150> Accessed on: Oct. 1, 2020.

Received in: June, 29, 2020
Accepted in: November, 25, 2020