Original Article

EFFECT OF SOYBEAN SEED TREATMENT ON THE POPULATION DYNAMICS OF *Pratylenchus brachyurus* UNDER WATER STRESS CONDITIONS

EFEITO DO TRATAMENTO DE SEMENTES DE SOJA NA DINÂMICA POPULACIONAL DE *Pratylenchus brachyurus* SOB CONDIÇÕES DE RESTRIÇÃO HÍDRICA

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ABSTRACT: The aim of the study was to evaluate the efficacy of seed treatment in the *Pratylenchus brachyurus* control and on the development parameters of soybean plants subjected to two water regimes. Seeds of the cultivar MSoy 7639 were treated with products containing imidacloprid + thiodicarb, pyraclostrobin + thiophanate-methyl + fipronil and abamectin + azoxystrobin + mefenoxam + fludioxonil + thiamethoxam. The plants were also subjected to two water regimes (absence and presence of water stress), maintaining 40 and 60% field capacity 15 days after nematode inoculation. At 60 days after inoculation, the aerial part and root fresh and dry weight were evaluated, together with the number of nematodes per gram of root and total nematodes (soil + root). Treating the seeds significantly reduced the population of *P. brachyurus*, irrespective of the water regime. Water stress also significantly reduced the total number of nematodes. In general, for a given water regime, the treatments did not affect vegetative development. However, plant development was significantly reduced under water stress, except for the treatment containing pyraclostrobin + thiophanate-methyl + fipronil. The conclusions were that seed treatment helped reduce populations of *P. brachyurus* and treatment with pyraclostrobin + thiophanate-methyl + fipronil resulted in beneficial changes in the soybean plants, increasing tolerance to water stress.

KEYWORDS: Root-lesion nematode. Seed treatment. Water stress.

INTRODUÇÃO

The root-lesion nematode, *Pratylenchus brachyurus* (GODFREY, 1929) Filipjev and Schuurmans Stekhoven 1941, has a wide host range by De Waele and Elsen (2002) and is fairly widely distributed in tropical regions, especially in Brazil. Over the last few years, it has caused significant and increasing damage and worrying losses to soybean crops, especially in the Brazilian Cerrado savanna regions.

In an attempt to reduce nematode populations, studies have been carried out to improve the integration of available control methods, with the aim of making the production process more rational, efficient and cost-effective (NOVARETTI, 1998). Since there are few cultivars available that are resistant to *P. brachyurus* and most crop species provide a good host for this nematode, using nematicides continues to be one of the most attractive control strategies in integrated management programs, to reduce nematode populations (KOENNING et al., 2004).

Shortage of water is the most important and frequent source of stress on soybean crops in Brazil. In various regions of the country, irregular rainfall patterns are common, affecting plant stands and growth. Treating the seeds with nematicide can render phytonematode control more effective as described by Bessi et al. (2010), avoiding damage in the initial stages of plant development and minimizing the effects of water stress by stimulating root development so that they penetrate deeper into the soil and can withstand water shortages, as well as reducing the risk of toxicity to humans and the environment.

Various studies on seed treatment with nematicides have been conducted to evaluate efficacy in reduction of the number of certain nematode species (BECKER; HOFER, 2004; KIEWNICK; GRIMM, 2005; LONG, 2005). However, studies on the use of seed treatment as a strategy for managing *P. brachyurus* are still quite rare. Therefore, the aim of this study was to evaluate the efficacy of soybean seed treatment in controlling root-lesion nematode, *P. brachyurus*, and the effects of different water regimes on soybean development parameters.

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MATERIAL AND METHODS

The experiment was conducted in the greenhouse in the Phytopathology Section of the Agronomy Faculty at the University of Rio Verde – FESURV from January to March 2011.

The *P. brachyurus* inocula used was taken from an infested soybean root collected in the municipality of Rio Verde, Goias State. To nematodes extraction in the laboratory, the infected soybean roots were washed, cut into pieces of around 1.0 cm, shredded in a blender and the suspension centrifuged, according to the method used by Coolen and D'Herde (1972). The number of nematodes per milliliter was estimated under the microscope using a Peters slide.

Soybean seed of the MSoy 7639 cultivar susceptible to *P. brachyurus* were treated with products containing imidacloprid + thiodicarb (600 mL 100 kg seeds^{-1}), pyraclostrobin + thiophanate-methyl + fipronil (150 mL 100 kg seeds^{-1}) and abamectin + azoxystrobin + mefenoxam + fludioxonil + thiamethoxam (500 mL 100 kg seeds^{-1}). Controls consisted of non-inoculated untreated seeds and inoculated untreated seeds.

Seed were treated manually according to the recommendations of the Brazilian Agriculture Ministry. The eight soybean seed for each treatment were sown immediately after nematicide application in plastic pots containing 13 kg of substrate (2:1 mix of soil and sand), disinfected beforehand using solarization as described by Guini (1997). Five days after seedling emergence, the plants were thinned to leave four plants per pot, and the seedlings inoculated with *P. brachyurus*. Two holes 2 cm deep and 1 cm from the stem of each seedling were created and the nematode suspension (2.5 ml) was inserted into each hole, providing an initial population (IP) of 500 nematodes per seedling, i.e. 2000 nematodes per pot.

Fifteen days after inoculation, the time required for second-stage juveniles (J2) to penetrate the roots, the maximum water retention capacity (field capacity) of the soil was determined in pots of the same volume as the experimental pots. They were filled with dry soil and weighed and water added to saturate the soil. After saturation, the pots were placed on a support to allow drainage of the excess water. Field capacity was calculated by comparing the weight of the dry soil in the pot with the hydrated pot soil. Next, all treatments were subjected to two different water regimes (no water stress and water stress). Under the regime with no water stress, volumes of 1000, 800 and 600 mL of water were added according to the plants daily water requirement, maintaining the soil moisture content at around 60% of field capacity. Under the water stress regime, half the above volumes of water were given to the plants, i.e. 500, 400 and 300 ml, enough to stress the plants without causing them to wilt permanently. These levels were maintained until the end of the experiment.

Sixty days after inoculation, the plants were carefully removed from the pots and the aerial parts separated from the root systems and plant height measured with a graduated rule. The aerial parts were weighed to obtain the aerial part fresh weight (APFW), and then packed in paper bags and kept at 50 ºC until their weights were constant to determine the aerial part dry weight (APDW). The root systems were washed in running water, placed on absorbent paper to eliminate the excess water, and then weighed to obtain the root fresh weight (RFW).

Nematodes in the soil were extracted using the methods of Jenkins (1964). The roots were cut and shredded for five seconds in a blender, as described previously. *Pratylenchus brachyurus* in the samples was quantified with a Peters slide under an inverted microscope. The number of *P. brachyurus* per gram of root (NGR) was determined, and then the total number of *P. brachyurus* (soil + root) (NT) was used to determine the final population (FP).

The experimental design was completely randomized with 10 replications in a 5 x 2 factorial with five treatments and two water regimes. Analysis of variance was performed and the means were compared using a 5% Tukey test, with the Sisvar 4.0 program as described by Ferreira (2000).

RESULTS AND DISCUSSION

Analysis of variance showed significant interaction between water stress and seed treatment for root fresh weight, total nematodes (soil + root) and number of nematodes per gram of root ($P \leq 0.05$).

Irrespective of water conditions, all the treatments applied significantly reduced the total number (FP) of *P. brachyurus* in comparison to the control without seed treatment and with nematodes (Table 1). However, comparing the two regimes, the water stress also had an adverse effect on nematode reproduction, since all treatments exhibited lower populations under water stress compared with plants with adequate water.

Table 1: Effect of interactions between water stress and soy seed treatments on the total population of *Pratylenchus brachyurus* (soil + root) (NT) and number of nematodes per gram of root (NGR) at 60 days after sowing.

Treatments	Water stress			
	Without	With	Without	With
	NT		NGR	
Control without nematodes[1]	0.00 c	0.00 c	0,00 c	0,00 d
Control with nematodes[1]	1.162.7 aA[2]	553.2 aB	25,67 aA	28,81 aA
Imidacloprid + thiodicarb	719.0 bA	346.9 bB	17,87 bA	19,16 bA
Pyraclostrobin + thiophanate-methyl + fipronil	512.2 bA	304.5 bB	18,34 bA	10,26 cB
Abamectin + azoxystrobin + mefenoxam + fludioxonil + thiamethoxam	598.9 bA	326.9 bB	17,88 bA	14,46 bcA
CV (%)	51.61		45.54	

[1]Without seed treatment; [2]Means followed by the same lowercase letter in the columns and uppercase letter in the rows, did not differ in the 5% Tukey test.

Evaluation of the number of nematodes per gram of root with no water stress, across all the seed treatments resulted in a lower number of *P. brachyurus* compared with the control and for both water regimes (Table 1). However, under water stress conditions, seed treatment with pyraclostrobin + thiophanate-methyl + fipronil resulted in the lowest number of *P. brachyurus* per gram of root compared with seed treatment using imidacloprid + thiodicarb, but did not differ statistically from the seed treatment with abamectin + azoxystrobin + mefenoxam + fludioxonil + thiamethoxam.

Comparing the two water regimes, only the treatment with pyraclostrobin + thiophanate-methyl + fipronil affected the population of *P. brachyurus* per gram of root when the plants were subjected to water stress, i.e. there were fewer nematodes per gram of root (Table 1).

Plant height at 60 days after inoculation and with no water stress was the greatest in the control without nematodes compared with seed treatment using pyraclostrobin + thiophanate-methyl + fipronil, but did not differ from the other treatments (Table 2). Under water stress conditions, plant height was the same irrespective of the treatment. Comparing the two regimes (water stress and no water stress), only the seed treatment with pyraclostrobin + thiophanate-methyl + fipronil did not affect plant height in the presence of water stress.

Similar results were obtained for aerial part fresh and dry weight and root fresh weight: only the plants treated with pyraclostrobin + thiophanate-methyl + fipronil remained unaffected by water stress (Table 2). For the other treatments, water stress significantly reduced aerial part fresh and dry weight and root fresh weight.

Results show that all the seed treatment chemical products evaluated reduced the total number of nematodes. This is important, since germination and seedling formation are key stages in plant development, during which nematode action can affect plant development and reduce capacity to withstand water stress. These results corroborate by Bessi et al. (2010), who obtained satisfactory results using abamectin to reduce populations of *Meloidogyne incognita* (Kofoid and White) Chitwood in cotton crop, probably in consequence of lower penetration or inhibition of juvenile activity in roots of the treated seeds. It is worth noting that abamectin effectively protects the cotton plant root system according to Lovato et al. (2007) and significantly reduces the number of plant galls and *M. incognita* in the root system (LOVATO, 2010; MONFORT et al., 2006). The above studies back up the findings of Cabrera et al. (2009), who observed that abamectin as a seed treatment is an effective way to limit nematode penetration into the roots without adversely affecting plant growth.

In a study of the performance of treatments containing imidacloprid + thiodicarb, Kubo et al. (2009) confirmed that it was possible to reduce *Rotylenchulus reniformis* (Linford and Oliveira) nematode in cotton plant roots and soil, increasing the initial development of the plant. Despite the scarcity of experimental results aimed at controlling *P. brachyurus* under various conditions, especially water stress, the data show that seed treatment with nematicides confers changes that benefit the plant, increasing tolerance even under water stress conditions and promoting better initial seedling development, possibly by inhibiting nematode penetration and colonization and thereby impairing the parasites ability to reproduce.

Table 2: Effect of interactions between water stress and seed treatment on plant height (PH), fresh weight (APFW) and dry weight (APDW) of the aerial part and root fresh weight (RFW) of soy plants with and without water stress at 60 days after sowing.

	Water stress							
Treatments[1]	Without	With	Without	With	Without	With	Without	With
	PH (cm)		APFW (g)		APDW (g)		RFW (g)	
T1	30.29 aA[2]	20.94 aB	36.43 aA	20.12 aB	8.00 aA	4.55 abB	47.29 aA	26.04 aB
T2	28.01abA	20.19 aB	30.45 aA	16.50 aB	6.41 bA	3.83 bB	48.90 aA	21.07 aB
T3	26.33 abA	21.00 aB	29.18 aA	20.73 aB	6.12 bA	4.72 abB	44.60 aA	20.55 aB
T4	24.31bA	23.90 aA	31.26 aA	24.65 aA	6.77 abA	5.67 aA	34.95 aA	31.88 aA
T5	28.52 abA	20.43 aB	36.49 aA	18.49 aB	8.25 aA	4.55 abB	38.04 aA	24.20 aB
CV (%)	17.71		31.57		20.97		34.58	

[1]Treatments: T1 - control without nematodes, T2 - control with nematodes, T3 - imidacloprid + thiodicarb, T4 - pyraclostrobin + thiophanate-methyl + fipronil, T5 - abamectin + azoxystrobin + mefenoxam + fludioxonil + thiamethoxam; [2]Means followed by the same lowercase letter in the columns and uppercase letter in the rows, did not differ in the 5% Tukey test.

The results obtained in terms of plant development show an increase in root fresh weight, a fundamental factor in the initial development and formation of plants in the field, improving the exploitation of nutrients and lowering predisposition to phytonematode attacks. Other authors have reported similar results in the greenhouse, such as taller plants, larger root systems and leaf area in soy grown from seed treated with pyraclostrobin + thiophanate-methyl + fipronil, both with and without water stress, Balardin et al. (2011). Coating maize seeds with fipronil (CAIXETA et al., 2010; ROYALTY, 1996) and rice, Caixeta et al. (2010) increased aerial part and root growth under water stress conditions. Products like fipronil (chlorine channels) that act on ion channels are associated with increased tolerance to biotic and abiotic stress, since these ions play a part in stomatal regulation and consequently in the response to water stress (KLEIN et al., 2004; KWAK et al., 2001).

In our study, the higher tolerance to water stress evidenced by plant development was obtained by treating seeds with pyraclostrobin + thiophanate-methyl + fipronil. In addition to the effect of fipronil described above, pyraclostrobin is a strobilurin whose ability to improve soy plant growth has already been documented (GROSSMANN and RETZLAFF, 1997; GROSSMANN et al., 1999; NASON et al., 2007). Ribeiro et al. (2010) succeeded in increased the development of soybean plant aerial parts and root systems using pyraclostrobin combined with thiophanate-methyl + fipronil and carbendazim + thiram. Some authors also report that applying pyraclostrobin to the leaves can have a positive effect on plant metabolism, Venâncio et al. (2003), improving the plant's ability to withstand water stress, Töfoli et al. (2003). However, there are few documented trials evaluating strobilurin seed treatments.

In most of the treatments applied in this study, water stress resulted in a reduction in aerial part and root fresh weight. Plants under water stress generally react by increasing the ratio of root to aerial part, since the aerial part is most affected, Smith and Cothren (1999). According to Taiz and Zeiger (2010), in some plants water stress not only restricts plant size but also the number of leaves since it reduces the number of branches as well as branch growth rates. However, a shortage of water in the soil can result in a drop in liquid photosynthesis, cutting the production of carbohydrates and in turn leading to a drop in plant biomass accumulation. In addition, when the plant is close to wilting permanently, photosynthesis is slow to fully recover, Mota (1983). A study by Levitt (1980) showed that plants adjust morphologically and anatomically to water stress, but these responses can vary according to the plant species, cultivar, plant development stage and water stress intensity.

The results of this study provide a basis for new research aimed at evaluating the use of seed treatment as a component in phytonematode management, especially under water stress conditions.

RESUMO: Objetivou-se avaliar a eficácia do tratamento de sementes no controle de *Pratylenchus brachyurus* e sobre parâmetros de desenvolvimento de plantas de soja submetidas a dois regimes hídricos. Sementes da cultivar MSoy 7639 foram tratadas com produtos contendo imidacloprido + tiodicarbe, piraclostrobina + tiofanato metílico + fipronil e abamectina + azoxistrobina + mefenoxam + fludioxinil + tiametoxam. As plantas foram submetidas a dois regimes hídricos, presença e ausência de estresse hídrico, mantendo-as com 40 e 60% da capacidade de campo, respectivamente, estabelecidos 15 dias após a inoculação do nematoide. Aos 60 dias após a inoculação, realizaram-se avaliações da massa fresca e seca da parte aérea, massa fresca da raiz, número de nematoides por grama de raiz, total de nematoides (solo+raiz) e fator de reprodução. Todos os tratamentos de sementes utilizados reduziram significativamente a população de *P. brachyurus*, independente do regime hídrico. A condição de estresse hídrico reduziu significativamente o número total de nematoides e o fator de reprodução, independente do tratamento. Dentro do mesmo regime hídrico, em geral, os tratamentos não afetaram o desenvolvimento vegetativo. Porém, o desenvolvimento das plantas foi significativamente reduzido sob estresse hídrico, com exceção do tratamento contendo piraclostrobina + tiofanato metílico + fipronil. Conclui-se que o tratamento de sementes teve efeito positivo na redução das populações de *P. brachyurus* e que o tratamento com piraclostrobina + tiofanato metílico + fipronil promoveu alterações benéficas às plantas de soja, aumentando sua tolerância ao estresse hídrico.

PALAVRAS CHAVE: Soja. Estresse hídrico. Nematoide das lesões. Tratamento de semente.

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