RESEARCH/INVESTIGACIÓN

POPULATION DYNAMICS OF THE ROOT LESION NEMATODE, *PRATYLENCHUS BRACHYURUS*, IN SOYBEAN FIELDS IN TOCANTINS STATE AND ITS EFFECT TO SOYBEAN YIELD

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ABSTRACT

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Pratylenchus brachyurus has become increasingly common in soybean fields throughout Brazil where yield loss assessments have reported reduction up to 30%. Currently, no soybean cultivars resistant to *P. brachyurus* have been identified, and management strategies include crop rotation with non-host crops, fallow, and the application of nematicides. The goals of this study were to examine the population dynamics of *P. brachyurus* in soybean fields throughout Tocantins state and in areas planted with off-season crops following soybean harvest in a crop succession scheme. *Pratylenchus brachyurus* was present in ca. 82% of samples with densities in soybean fields ranging from 23 to 20,400 nematodes per 200 cm^3 soil or 10 g root samples. In two sites, the mean nematode density was higher within infestation foci, which were characterized by poor soybean growth, compared to those from outside infestation foci, with an overall reduction of 44.3% in plant height and 39.7% in pod numbers. Following soybean harvest in areas planted with off-season crops including maize, sorghum, millet, crotalaria as well as an area maintained as a fallow treatment, the mean density of *P. brachyurus* ranged from 122 to 504 individuals per 10 g root sample and from 3 to 96 per 200 cm^3 soil. Overall, the mean density of nematodes did not differ significantly among plant species and all crops used in the succession scheme allowed *P. brachyurus* multiplication. In summary, off-season cultivation with the crops used in this study is not recommended for management of *P. brachyurus* in soybean, but the use of fallow or non-hosts may be helpful in lowering the population density of *P. brachyurus* in soybean fields.

Key words: *Glycines max*, management, nematode, population survey, yield loss.

RESUMO

Lima, F. S. D. O, G. R. D. Santos, S. R. Nogueira , P. R. R. D. Santos, e V. R. Correa. 2015. Dinâmica populacional do nematoide das lesões radiculares *Pratylenchus brachyurus* em campos de soja no Tocantins e seu efeito na produção. Nematropica 45:170-177.

Pratylenchus brachyurus tem se tornado cada vez mais frequente em campos de soja em todo Brasil, com perdas na producção estimada em 30%. Atualmente, não há relatos de cultivares de soja resistentes a *P. brachyurus* e as estratégias de controle incluem rotação com culturas não hospedeiras, pousio, além do uso de nematicidas. Este trabalho teve como objetivos avaliar a dinâmica populacional de *P. brachyurus*, em áreas de soja no estado do Tocantins, e em áreas plantadas com culturas de safrinha, em esquema de sucessão, após colheita de soja. As densidades de *P. brachyurus* observadas em campos de soja, em onze municípios do estado do Tocantins, variaram de 23 a 20.400 nematoides por amostras de 200 cc de solo ou 10 g de raízes. Em dois locais, a média da densidade de nematoides foi maior, com desenvolvimento menor da soja, em amostras de solo e raízes tomadas dentro de reboleiras com sintomas do nematoide, comparados com amostras de parcelas fora das reboleiras; foi observada uma redução geral de 44,3% na altura das plantas e 39,7% no número de vagens. Em áreas plantadas com culturas de entressafra, previamente cultivadas com soja, incluindo milho, sorgo, milheto, crotalária, além de uma área de pousio como tratamento, a densidade média de *P. brachyurus* variou entre 122 e 504 espécimes por 10 g de raiz e entre 3 e 96 por 200 cc de solo. No geral, a densidade média não diferiu estatisticamente entre as espécies testadas e todas as culturas utilizadas no esquema de sucessão permitiram a multiplicação de *P.*

brachyurus. Em conclusão, o cultivo dessas espécies vegetais usadas neste estudo não é recomendado para o manejo de *P. brachyurus* em campos de soja. No entanto, o uso de culturas não hospedeiras ou a utilização do sistema de pousio podem ajudar a diminuir o nível populacional de *P. brachyurus* em áreas de soja.

Palavras-chave: fitonematoides, *Glycines max*, levantamento populacional, manejo.

INTRODUCTION

Brazil is the largest producer and exporter of soybeans (*Glycine max* L. Merr) worldwide, making this crop an important commodity for its economy. Over 50% of soybean in Brazil is produced in the Cerrado region, with a recent increase in production between 100% and 600% in the states of Tocantins, Maranhão, Piauí, Bahia, and Goiás. Tocantins state is considered the largest grain producer in this region, producing 1.4 million tons of soybeans in an area of 748 thousand hectares in 2012 to 2013 (Seagro, 2014).

Tocantins state has a climate favorable for soybean production and is strategically located, so the potential for export is high. Moreover, with the implementation of the 'Northern Hallway Export Program', covering the Cerrado region of Southwest Piauí, Southern Maranhão, North and Southeast of Tocantins, commercial opportunities for soybean production in this region are expected to expand due to improvements in infrastructure and transportation.

The expansion in soybean production in the Cerrado region has contributed to intensive agriculture, leading to agronomic challenges, including nematode diseases. Currently, more than 100 nematode species within 50 genera have been reported causing damage to soybean (Ferraz, 1999). In Brazil, the most important nematode species associated with soybean are the root-knot nematodes, *Meloidogyne javanica* (Treub, 1985) Chitwood, 1949 and *M. incognita* (Kofoid and White, 1919) Chitwood, 1949, soybean cyst nematode *Heterodera glycines* Ichinohe, 1952, root lesion nematode, *Pratylenchus brachyurus* (Godfrey, 1929) Filipjev and Stekhoven, 1941), *Tubixaba tuxaua* Monteiro and Lordello, 1980, and the reniform nematode, *Rotylenchulus reniformis* Lindford and Oliveira, 1940 (Roese *et al.*, 2001; Franzener *et al.*, 2005; Dias *et al.*, 2007; Santana *et al.*, 2009).

Among these nematode species, the root lesion nematode, *P. brachyurus*, is probably one of the most important, especially in the Cerrado region. Recently, its incidence has been rising with overall losses estimated at ca. 10-30%, especially in sandy soils receiving irregular rainfall (Ferraz, 2006; Panorama Rural, 2010; Plantio Direto, 2014, Waldir P. Dias, Embrapa Soybean, Campo Grande, MT, personal communication). In addition, there are frequent reports of yield losses and economic damage in several soybean fields across Tocantins state, with special concern with damage caused by *P. brachyurus* (Lima Neto, 2009).

Pratylenchus brachyurus directly affects soybean growth and yield. It causes root necrosis, which affects water and nutrient uptake. Pathogenicity assays in soybeans indicated that this nematode is well adapted to parasitism in which high populations present in soil usually are not enough to kill host plant. However, damage thresholds are very variable and depend on a combination of *Pratylenchus* species and host plants, ranging from 0.05 to 30 individuals per cc soil (Castillo and Vovlas, 2007).

Pratylenchus brachyurus is a migratory endoparasitic nematode that moves intercellularly through the root cortex, disrupting and destroying cells, facilitating secondary infection by fungi and bacterial pathogens (Castillo and Vovlas, 2007). Symptoms associated with *P. brachyurus* infection are non-specific and can easily be missed or mistaken for symptoms caused by other pathogens, nutritional deficiencies and water stress. Such symptoms include root necrosis, discolored roots (reddish to dark brown), stunted plants with chlorosis and wilting – symptoms that may lead to crop damage and yield loss (Ferraz, 1999; Castillo and Vovlas, 2007).

Economically important nematode species are usually present in Cerrado native soils at low population densities. However, with increased use of soybean and cotton monocultures, their populations have increased substantially (Goulart, 2008; Lima Neto, 2009). The withdrawal of most chemical nematicides from the market due to concern for human and environmental side effects has pressed the need for new control strategies, including genetic control of nematodes. Host resistance has been explored, but no *P. brachyurus*-resistant soybean cultivars have been identified so far. Breeding resistant cultivars against *P. brachyurus* is difficult due to the fact that this nematode is polyphagous and lacks an intimate interaction with their hosts. Hence, current management recommendations for reducing *P. brachyurus* in soybeans include crop rotation or succession with non-hosts as well as fallow.

The goals of this research were to examine the population dynamics of *P. brachyurus* in soybean

fields in Tocantins state and in a crop succession scheme with off-season species planted following soybean harvest through the assessment of nematode survey and yield loss in infested areas.

MATERIALS AND METHODS

During cropping seasons of 2009 to 2011, from several soybean samples collected in soybean fields and submitted voluntarily by soybean growers to the Nematode Assay Laboratory (Faculdade Católica do Tocantins) for nematode analysis, 33 samples from 11 counties in Tocantins state were further examined for the presence of nematodes. Soybean samples were taken from January to March at the beginning of soybean flowering stage or during seed filling (R1-R4 reproductive stages). Most soybean fields sampled were characterized by a sandy Red Latosol. Samples were collected with a shovel around plants showing nematode symptoms within 20-cm depth. A total of fifteen 300 cc soil samples or roots were mixed to make a composite sample of 200 cc soil and 10 g roots. Three composite samples for each county were used to estimate and identify the most prevalent nematode species.

Nematodes were extracted from soil using the flotation, sedimentation and centrifugation method (Jenkins, 1964) or from roots according to Coolen and D'Herde (1972) and Jenkins (1964). Extracted nematodes were killed at 55ºC for 30 sec, fixed in Golden solution (3% formalin final volume) (Hopper, 1970), counted and identified to species level using morphological characters (Handoo and Golden, 1989). Based on population densities from the survey, two sites in Tocantins state were selected for additional studies to examine the population dynamics of *P. brachyurus*. One site was in Porto Nacional, and one site was in Silvanópolis. In Porto Nacional, the goal was to determine soybean yield losses due to *P. brachyurus* in an area that had been planted with soybean for five to six consecutive cropping seasons. The Silvanópolis site was used to evaluate the effect of off-season crops planted after soybean on *P. brachyurus* populations.

In Porto Nacional (experiments I and II) soybean cv. 'MONSOY9144' was planted on November 25 of 2010 and 2013. Eighty days post planting, five areas (foci) of apparent *P. brachyurus* infestation were randomly chosen and assigned to a block. Each block was divided into two plots (4 m^2), one inside and one outside the infestation focus. In each plot, three soil samples of 200 cc and 10 g of roots were systematically taken in a zigzag pattern to estimate *P. brachyurus* population densities. Samples were taken around plant within a 20-cm depth with a soil core of 2.5-cm diameter. All samples were collected from soybean plants only, normally during flowering stage or early during seed filling stage. The soil type was mostly characterized by sandy Red Latosol. Nematodes were extracted from soil using the flotation, sedimentation, and centrifugation method (Jenkins, 1964) or from roots according to Coolen and D'Herde (1972) and Jenkins (1964), killed (55ºC for 30 sec), fixed in Golden solution, counted and identified to species level using morphological characters as mentioned above (Handoo and Golden, 1989). Additional parameters such as soybean growth (plant height, stem diameter, internode and leaf numbers, dry weight of shoot, root, and stem) and production (pod numbers and seed weight) were analyzed.

At the Silvanópolis site (experiment III), an area of 240-m^2 infested with *P. brachyurus* was planted with soybean and off-season rotational crops following soybean harvest. The crops included soybean (cv. MONSOY 9144), *Crotalaria juncea*, a maize hybrid ('Pioneer 30F35H'), a sorghum hybrid ('DKB599'), and pearl millet ('ADR 7010'). A fallow treatment was also included. Prior and after soybean harvest, the population density of *P. brachyurus* was determined. The 240 m^2 area was further divided into 3 subareas of 80 m^2 and a total of 20 subsamples of roots or soil per subarea were collected with a soil core of 2.5 cm diameter in a zigzag pattern and combined into a composite sample. Nematodes were extracted, fixed, counted, and identified to species level using morphological characters as mentioned above. Crops were planted in a randomized block design with four replicates in March 20, 2011, and sampled 60 d later. Three soil subsamples were collected to a depth of 15 cm in the three central rows of each plot using a soil core of 2.5-cm diameter. Samples were pooled to make a composite sample of 200 cc for nematode assay. Plants were collected from the same points, to determine nematode population density in 10 g of roots, using methods described above.

Data were submitted to analysis of variance (one-way ANOVA), and the means separated using the Tukey's test ($P < 0.05$). Data were also transformed as log (x + 1) and submitted to regression analysis.

RESULTS

Results from this survey showed that *P. brachyurus* was the most prevalent nematode species found associated with soybeans, with more than 95% of the specimens identified. Densities of *P. brachyurus* observed in soybean fields in several counties throughout Tocantins state ranged from 23 to 20,400 nematodes per sample (Table 1). Other nematode species found included *P. zeae*, *Helicotylenchus* spp., *Criconemella* spp., and *Meloidogyne* spp.

Table 1. Population densities of *Pratylenchus brachyurus* found in soybean fields in Tocantins state, Brazil, during 2009-2011.

	No. of *P. brachyurus*	
Counties[y]	10 g of roots[z]	200 cc of soil[z]
Santa Rosa	24 ± 2.33	12 ± 0.88
Porto Nacional	5,482 ± 289.24	139 ± 14.50
Ipueiras	2,669 ± 112.49	13 ± 1.86
Chapada de Natividade	366 ± 27.72	44 ± 3.48
Rio dos Bois	2,157 ± 93.87	56 ± 6.84
Brejinho de Nazaré	1,452 ± 56.44	63 ± 6.67
Buritirana	940 ± 32.17	34 ± 4.48
Tupirama	890 ± 23.63	45 ± 4.09
Aparecida do Rio Negro	509 ± 30.14	64 ± 3.76
Itacajá	3,852 ± 56.29	18 ± 3.92
Silvanópolis	1,020 ± 40.41	38 ± 4.41
Mean	1.757.36	47.82

[y]Counties located in Tocantins state, Brazil.
[z]Means (n = 3) ± standard error of *P. brachyurus* densities from three independent soybean sampling sites.

Table 2. Soybean yield inside and outside *Pratylenchus brachyurus* infestation foci in a field in Silvanópolis, Tocantins, Brazil, during the cropping season of 2010/2011.

Treatments	*P. brachyurus* (10 g of roots)	*P. brachyurus* (200 cc of soil)	Plant height (cm)	No. of pods	Weight (g) (100 seeds)
Within infestation foci[y]					
1	4,428	24	40.9	54.0	170
2	3,845	84	44.0	69.4	270
3	2,898	400	52.6	68.3	171
4	1,545	27	40.8	42.0	105
5	3,673	0	40.0	45.2	113
Mean[z]	3,278[z] a	107 a	43.6 a	55.7 a	165.8 a
Outside infestation foci					
1	150	7	89.0	83.0	240
2	279	0	63.4	123.0	310
3	77	32	81.2	76.0	191
4	390	38	80.8	112.4	282
5	27	0	84.8	85.2	214
Mean	185[z] b	15 b	79.8 b	96 b	247.4 b
Yield reduction (%)	-	-	44.3	39.7	33

[y] Numbers 1-5 represent replicates.
[z]Means (n = 5) followed by different letters within columns are significantly different according to Tukey's test ($P < 0.05$).

In the first experiment, soybean plants showed typical symptoms caused by *P. brachyurus*, including uneven plant growth with darkened, necrotic roots and fewer secondary roots. The population density of *P. brachyurus* in soil and root samples taken within infestation foci was higher than those from plots outside infestation foci (Table 2). Densities of *P. brachyurus* inside infestation foci were around 14 times greater than outside the foci. Plants inside the infestation foci were 44.3% shorter and produced 39.7% fewer pods, on average, than asymptomatic plants outside the foci. Overall, there was a negative correlation of *P. brachyurus* density with plant height (R^2 = -0.78, P = 0.001), pod numbers (R^2 = -0.48, P = 0.03), root weight (R^2 = -0.45, P = 0.05), and seed weight (R^2 = -0.33, P = 0.1), although not statistically significant for this late variable (Table 2).

Data collected during 2012/2013 harvest (experiment II) also showed the same trend. There was a negative correlation between *P. brachyurus* density and soybean (cv. MONSOY 9144) growth (R^2 = -0.42, P = 0.05) and yield (R^2 = -0.48, P = 0.03) (Table 3). The average values of internode numbers, stem diameter, leaf numbers, dry weight of roots, leaves, stem, pods and shoot, pod numbers, and plant height within infestation foci were all statistically lower than soybean outside infestation foci (Table 3).

Planting non-host crops after soybean did not reduce population densities of *P. brachyurus* (Table 4). *Pratylenchus brachyurus* density in crotalaria (*C. juncea*) and on maize cv. Pionner 30F35H was 126 and 504 individuals per 10 g roots, respectively, while in sorghum DKB599 and on pearl millet ADR 7010 was 122 and 309 individuals per 10 g roots, respectively.

DISCUSSION

Our study confirmed that *P. brachyurus* is common in soybean fields and associated with yield loss in the Tocantins state of the Cerrado region of Brazil. Previous nematode surveys carried out recently in other regions, such as Goiás and Mato Grosso do Sul states, showed that increased incidence of this nematode is positively correlated with a drop in soybean yield. For example, in Chapadão do Sul, Mato Grosso do Sul state, soybean yield was normally around 2,600 kg/ha, while during cropping season of 2008/2009 it dropped to 2,400, and to 1,850 during 2009/2010 (Panorama Rural, 2010, Waldir P. Dias, Embrapa Soybean, personal communication). In soybean fields in the west region of Brazil there are frequent reports of reduced yields up to 30% due to *P. brachyurus* attack (Goulart, 2008, Franchini *et al.*, 2008; Waldir P. Dias, personal communication).

Results of a study carried out in 2011/2012 in Vera-Mato Grosso state (Franchini *et al.*, 2008) indicated a highly negative correlation between soybean yield and nematode population, with 50 kg loss in yield for every 82 nematodes per gram of soybean roots. Overall, yield losses ranged from 50 to 1,400 kg/ha, with an average of 600 kg/ha (21%) yield reduction.

Ferraz (1995) showed a significant reduction in the growth of three soybean cultivars in the greenhouse under conditions favorable to soybean growth. At an inoculum level of 5,000 nematodes, there was a reduction in root fresh weight of 13.9 to 41.8% and shoot weight of 14.1 to 33.3% compared to the nematode-free control. Our results show that the impact of *P. brachyurus* is severe in fields with sandy soil causing reductions in plant height (ca. 82%), dry weight of seeds (85%), dry weight of stems (81%), and pod numbers (39.7%) in nematode infestation foci compared with plants outside these foci.

All crops used in the succession scheme were hosts of *P. brachyurus*, allowing its multiplication. Our results disagree with those of Borges (2009) who reported that pearl millet cultivars, including ADR7010 that were inoculated with *P. brachyurus* under greenhouse conditions, showed a reproduction factor of only 0.2.

Our data also showed that crop succession of soybean with either maize and or sorghum favored *P. brachyurus* multiplication. Crotalaria is a host to *P. brachyurus*, as evidenced by a population of 126 individuals per 10 g of roots. Several studies have shown that *Crotalaria juncea* is effective in controlling root-knot nematodes (Huang and Silva, 1980; Silva *et al.*, 1989; Moura, 1991; Araya and Caswell-Chen, 1992; Moura, 1995; Santana *et al.*, 2003). However, there are few studies considering the reaction of *C. juncea* to *P. brachyurus*, particularly under field conditions. According to Machado *et al.* (2007), *C. spectabilis* and *C. breviflora* are both non-hosts to *P. brachyurus*.

Fallow may be an alternative to planting crops off-season, but in this study, there was no reduction in *P. brachyurus* density in the crop succession soybean-fallow-soybean. It is possible that the 60-d period studied in our experiment was not a long enough time to allow nematode density to decrease. According to Mani (1999), soil plowing might increase the efficacy of fallow by exposing nematodes to sunlight and desiccation. However, the impact of plowing and fallowing can have negative impacts on soil properties or even increase *P. brachyurus* densities following soybean cropping (Franchini *et al.*, 2008).

It is important to assess *P. brachyurus* population dynamics in soybean fields across several cropping years in order to monitor its fluctuation, and help decide the best cropping system and other control

Table 3. Yield reduction in soybean inside and outside *Pratylenchus brachyurus* infestation foci in a field in Porto Nacional, Tocantins, Brazil during the cropping season of 2012/2013.

Treatments	No. of internodes	Stem diameter (mm)	No. of leaves	Root weight (g)	Leaves weight (g)	Stem weight (g)	Pods weight (g)	Shoot weight (g)	No. of pods	Plant height (cm)	No. of nematodes (roots)	No. of nematodes (soil)
Within infestation foci												
1	13[y]	6	23	4.70	9.96	4.51	2.96	17.43	31.36	30	1,385	422
2	13	3	21	1.38	7.50	1.71	0.94	10.15	30.72	13	2,172	182
3	13	4	16	1.57	5.85	1.47	0.52	7.84	27.58	8	1,232	247
4	13	4	19	1.83	7.24	1.41	0.56	9.22	25.76	11	1,322	175
5	13	4	16	3.93	7.43	1.96	0.64	10.03	29.70	10	1,047	700
Mean[z]	13 b	4.2 b	19 b	2.68 b	7.6 b	2.21 b	1.12 b	10.93 b	29.02 b	14.56 b	1,431 b	422 b
Outside infestation foci												
1	16	8	46	3.82	15.12	10.02	7.83	32.97	48.08	82	1,461	260
2	15	11	69	6.23	30.85	16.88	16.10	63.83	47.06	129	474	82
3	16	7	42	2.64	12.01	8.27	4.73	25.01	48.16	68	744	14
4	16	8	39	3.64	12.08	10.91	3.86	26.85	53.16	53	304	490
5	15	7	43	3.69	14.47	12.35	5.44	32.25	48.86	76	565	360
Mean	15.5 a	8.08 a	47.64 a	4 a	16.91 a	11.69 a	7.59 a	36.18 a	49.06 a	81.32 a	709 a	260 a
Reduction (%)	16.12	48	60.14	33	55	81	85	69.8	40.8	82.09	-	

[y]Numbers 1-5 represent replicates.

[z]Means ($n = 5$) followed by different letters within columns are significantly different according to Tukey's test ($P < 0.05$).

Table 4. Population densities of *Pratylenchus brachyurus* in a crop succession scheme post soybean cultivation during 2010/2011 in Porto Nacional, Tocantins.

Treatments	*P. brachyurus* (*Pi*[y]) (200 cc soil)	*P. brachyurus* (10 g roots)	*P. brachyurus* (200 cc soil)
Soybean-crotalaria (*Crotalaria juncea*)[z]	12 ± 5.40	126 ± 61.93	23 ± 15.58
Soybean-maize Pioneer 30F35H	39 ± 8.45	504 ± 195.45	21 ± 8.44
Soyben-sorghum DKB599	6 ± 2.50	122 ± 55.44	3 ± 1.78
Soybean-pearl milet ADR 7010	4 ± 1.50	309 ± 204.62	96 ± 60.19
Soybean-fallow	21 ± 6.45	-	54 ± 40.74
Mean	16	265	39.4

[y]Initial population density of *P. brachyurus* following soybean harvest.
[z]Treatment means ($n = 4$) were not significantly different according to Tukey's test ($P < 0.05$).

strategies for this nematode. In fields with medium to high densities of *P. brachyurus*, appropriate management methods should be adopted as part of a continuous program of pest management in order to positively impact soybean yield. In summary, off-season cultivation with maize or sorghum is not recommended with soybean, at least temporarily, and use of fallow or a non-host as cover crop to manage *P. brachyurus* in soybean field is suggested.

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