

## EFFECT OF SOYBEAN SEED TREATMENT WITH ABAMECTIN AND THIABENDAZOLE ON *HETERODERA GLYCINES*

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### ABSTRACT

Vitti, A. J., U. da R. Rezende Neto, F. G. de Araújo, L. C. Santos, K. A. G. Barbosa, M. R. da Rocha. 2014. Effect of soybean seed treatment with abamectin and thiabendazole on *Heterodera glycines*. *Nematropica* 44:74-80.

The option of chemical control of the soybean cyst nematode (SCN) with traditional nematicides has been unviable, but seed treatment nematicides could be an important tool for management. Abamectin applied at 0, 30, 40, and 50 g ha<sup>-1</sup> as a seed treatment together with or without thiabendazole at 20 g ha<sup>-1</sup> was studied on a SCN-resistant and a susceptible soybean cultivar. Abamectin treatment reduced the population density of females and cysts on the susceptible cultivar BRSGO Luziânia. For combined seed treatments, thiabendazole and abamectin should only be associated at concentrations of abamectin above 40 g ha<sup>-1</sup>. Combinations at lower rates or thiabendazole without abamectin resulted in a nematode population increase. The resistant cultivar BRSGO Ipameri yielded the highest, but seed treatment had no effect on yield.

*Key words:* chemical control, *Glycine max*, resistance, soybean cyst nematode.

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### RESUMO

Vitti, A. J., U. da R. Rezende Neto, F. G. de Araújo, L. C. Santos, K. A. G. Barbosa, M. R. da Rocha, 2014. Efeito do tratamento de sementes de soja com abamectina e tiabendazol em *Heterodera glycines*. *Nematropica* 44:74-80.

A opção do controle químico da densidade populacional do nematoide de cisto da soja (NCS, *Heterodera glycines*) tem sido inviável, mas o tratamento de sementes pode ser uma importante ferramenta para manejo. Abamectina foi utilizada para tratamento de sementes nas doses de 0, 30, 40 e 50 g ha<sup>-1</sup>, com ou sem thiabendazole (20 g ha<sup>-1</sup>), em cultivares de soja resistente e suscetível ao NCS. O tratamento com abamectina reduziu a densidade populacional de fêmeas e cistos na cultivar suscetível BRSGO Luziânia. Thiabendazole e abamectina somente devem associados para o tratamento de sementes a concentrações de abamectina acima de 40 g ha<sup>-1</sup>. Combinações a doses mais baixas ou sem abamectina resultaram em aumento populacional do nematoide. A cultivar resistente BRSGO Ipameri apresentou maior produtividade mas o tratamento de sementes não teve efeito sobre a produtividade.

*Palabras clave:* controle químico, *Glycine max*, nematoide de cisto da soja, resistência.

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The soybean cyst nematode, *Heterodera glycines* Ichinohe, 1952 (SCN), is widespread in soybean production fields in Brazil. The pest is estimated to occur on approximately 3.0 million hectares (Dias *et al.*, 2010), and threatens the soybean crop in Brazil (Embrapa, 2011). Its management is based on crop rotation and the use of resistant cultivars. Unfortunately, the level of SCN resistance in soybean cultivars varies. Most of these cultivars were derived from crosses that included the genotype PI88788, which does not confer durable resistance (Faghihi *et al.*, 2007). Even in cultivars with higher resistance

levels, some nematode reproduction does occur. This selection pressure can lead to development of virulent types that further threaten soybean production (Von Qualen, 2007).

Although genetic variability of *H. glycines* is higher in Brazil than in the United States (Dias *et al.*, 2006), there are no reports of *H. glycines* race shifts in Brazil. As suggested by Colgrove *et al.* (2002), Dong *et al.* (2005), and Faghihi *et al.* (2007), however, it might be a matter of time before these race shifts appear in the country. Therefore, alternative control measures that suppress SCN population density and

reduce selection pressure are needed. Eggs produced by *H. glycines* may remain inside the female body that, upon death, becomes a cyst with a hardened protective wall (Turner and Rowe, 2006). Consequently, conventional soil-applied nematicides may be only marginally effective. Seed treatment with nematicides may be an alternative tool in suppressing or reducing the nematode population during the early phase of the crop.

Abamectin is a nematicide/insecticide of the chemical group of avermectins that has shown positive results in the control of various nematodes when applied at relatively low rates (Monfort *et al.*, 2006; Faske and Starr, 2006; Moreira *et al.*, 2008). Silva *et al.* (2004) observed that 0.42  $\mu\text{g ml}^{-1}$  abamectin was sufficient to immobilize and kill second-stage juveniles (J2) of *Meloidogyne incognita* in vitro. Similar results were reported by Faske and Star (2006) in laboratory bioassays, where sublethal concentrations of 1.56 - 0.39  $\mu\text{g ml}^{-1}$  for *M. incognita* and 32.9 - 8.2  $\mu\text{g ml}^{-1}$  for *Rotylenchulus reniformis* reduced the infectivity of these nematode species on roots of tomato (*Solanum lycopersicum*) plants.

Although abamectin was tested successfully for nematode control in several application forms, including as a foliar spray (Pedrozo *et al.*, 1999), root treatment and injection into the pseudostem of banana (Jansson and Rabatin, 1998), the seed treatment seems to be the most satisfactory application form of this product (Faske and Star, 2006; Monfort *et al.*, 2006).

Thiabendazole, a fungicide of the benzimidazole group, is used as a broad-spectrum anthelmintic for several animal species and in humans. It is also a powerful systemic fungicide used in leaf sprays and postharvest fruit treatments (Oliveira *et al.*, 2002; Fischer *et al.*, 2008) as well as seed treatments (Moraes *et al.*, 2003; Nerbass *et al.*, 2008). In agriculture, McLeod (1973) showed that thiabendazole (at 2.5, 5, 10, and 20 ppm) inhibited the multiplication of *Aphelenchoides composticola* and *Ditylenchus myceliophagus* for more than 3 wk during mushroom (*Agaricus bisporus*) cultivation. However, the results were attributed to possible metabolites produced in plants and not to a direct toxicity of the product to nematodes.

Although other authors have tested abamectin on *H. glycines*, the combination of abamectin and thiabendazole as seed treatment has not been reported. The purpose of this study was to evaluate the effect of abamectin and thiabendazole as a soybean seed treatment on the population density of soybean cyst nematode under field and greenhouse conditions.

### Field Study

A field experiment was conducted in Campo Alegre, Goiás, Brazil, during the 2006 and 2007 growing seasons. The field had a severe infestation of *H. glycines*, race 3. Plots were sampled before planting

the experiment to assess the initial population, and the results indicated a relatively uniform population density with an approximate average of 146 cysts and 3,000 eggs/100  $\text{cm}^3$  soil.

Seeds of the SCN race 3-resistant cultivar, BRSGO Ipameri, and susceptible BRSGO Luziânia, were treated with abamectin (Avicta® 500 FS (500 g L<sup>-1</sup> abamectin) Syngenta, Greensboro, NC) at rates of 0, 30, 40, and 50 g ha<sup>-1</sup>, with or without the addition of 20 g thiabendazole ha<sup>-1</sup> (Tecto® SC (485 g L<sup>-1</sup> thiabendazole) Syngenta, Greensboro, NC).

The experiment was arranged in a randomized block design, in a factorial (2 cultivars  $\times$  4 abamectin rates  $\times$  2 thiabendazole rates) with four replications. Each plot consisted of 6 rows 6 m in length, spaced 0.45-m apart. Planting rate was 15 seeds m<sup>-1</sup> at an average depth of 3 cm.

After soil preparation, the plots were furrowed and fertilized with 300 kg ha<sup>-1</sup> of 0-20-20 N-P-K prior to sowing. Weeds were controlled by Dual Gold® herbicide applications (S-metolachlor - 960 g a.i. L<sup>-1</sup> EC, Syngenta, Greensboro, NC) at 1,750 ml ha<sup>-1</sup> before emergence and Fusiflex (fomesafen + fluazifop-p-butyl - 125 + 125 g a.i. L<sup>-1</sup> EC, Syngenta, Greensboro, NC) at 1,600 ml ha<sup>-1</sup> after emergence in the growth stage of three trifoliate leaves. A bedbug (*Nezara viridula* and *Piezodorus guildinii*) infestation was controlled by one application of Engeo Pleno (thiamethoxam + lambda-cyhalothrin - 141 + 106 g a.i. L<sup>-1</sup> CS, Syngenta) at 200 ml ha<sup>-1</sup> at stage R4 (Fehr and Caviness, 1977) when the population reached the level of 0.5 bugs per 1 m  $\times$  1 m drop cloth. Soybean rust was controlled by two applications of the fungicide Piori Xtra (azoxystrobin + cyproconazole - 200 + 80 g a.i. L<sup>-1</sup> CS, Syngenta, Greensboro, NC) at a dose of 300 ml ha<sup>-1</sup> plus 600 ml ha<sup>-1</sup> of adjuvant Nimbus (paraffin mineral oil, Syngenta) at the stages R2 and R5.3 (Fehr and Caviness, 1977).

The number of SCN females on roots and the number of cysts in the rhizosphere as well as the number of eggs per female were determined at 30, 45, and 60 days after sowing (DAS). Evaluations of females on the roots were based on collection of the roots of four plants randomly collected per plot. The *H. glycines* females were removed by vigorously washing the roots on a set of sieves (0.84-mm on 0.25-mm pore size). The females trapped in the 0.25-mm pore sieve were collected and the suspension filtered through filter paper (Southey, 1986). The number of females was immediately determined by counting under a stereomicroscope (30 $\times$  magnification).

Each plot was sampled by collecting four soil subsamples from the root zone at 0 to 15-cm depth with the use of a small shovel. The cysts were extracted from a 100  $\text{cm}^3$  soil sample by suspension in water and recovered from nested 0.84/0.25 mm pore size sieves (Southey, 1986). Number of cysts was determined by counting under stereomicroscope (30  $\times$  magnification) and presented as cysts of *H. glycines*/100  $\text{cm}^3$  of soil.

The number of eggs per female was determined in 10 females randomly chosen from those extracted from the roots. Females were crushed by a glass rod on a 0.15/0.038 mm pore size sieve set. After washing under tap water, the released eggs and J2 were collected from the 0.038 sieve, re-suspended in water, and the concentration was measured in a Peters chamber with a stereomicroscope. For the correlation between the volume of the suspension obtained and the number of eggs and J2 per milliliter, the average number of eggs per female was determined.

At the end of the crop cycle, plants were collected from 4 m of the 2 central rows of each plot to determine the yield. Weight and grain moisture per plot were determined. Yield was reported in kg/ha, adjusted to 13% moisture.

### Greenhouse Study

Seeds of BRSGO Ipameri and BRSGO Luziânia received the same treatments as in the field experiment described above. Two seeds per pot were sown 1.5-cm deep in clay pots containing 1,300 cm<sup>3</sup> of a 1:1 (v/v) soil-sand mixture autoclaved at 120°C for 20 min.

After seedling emergence, a suspension containing eggs and J2 of *H. glycines* race 3 was deposited into two holes (diameter 0.5 cm, depth 1.5 cm) in the soil around the seedling. The suspension was calibrated so that each pot received approximately 4,000 eggs and J2 in the mixture.

The experiment was arranged in a completely randomized 2 × 4 × 2 factorial design (2 soybean cultivars × 4 abamectin rates × 2 thiabendazol rates) with 5 replications. Soil and air temperatures were monitored daily. The average air temperature during the period of the experiment was 29.8°C, ranging from 27°C to 35°C. Soil temperature ranged from 25°C to 30°C and averaged 26.2°C. Air temperature was taken from a maximum and minimum thermometer and soil temperature from a geothermometer. Irrigation was provided as needed by pouring water into each pot with a beaker, avoiding water excess.

Evaluations were performed 30 d after inoculation by determining the population of females on the roots, cysts in the substrate, and number of eggs per female, according to the above methodology.

Data were transformed when necessary, according to test of homogeneity of variance (Box and Cox, 1964). The data for both studies were subjected to analysis of variance and means compared by the F test at 5% probability level. In the case of significant differences between the abamectin rates, regression analyses were performed. Since *H. glycines* population density was evaluated three different times (30, 45, and 60 DAS), regression analyses were also performed when significant differences were found over time. Statistical analyses were done with the use of the software package SISVAR (Ferreira, 2000).

Seed treatment had no effect on seedling emergence

in the field. Chlorosis and stunted growth were noticed on the susceptible cultivar BRSGO Luziânia during the evaluation of plant stand and plant height as similarly described by Lilley *et al.* (2005) and Dias *et al.* (2006). Although *H. glycines* must have already established and developed at 30 DAS, differences in plant height were only observed 45 DAS between the resistant and susceptible cultivar. The resistant cultivar BRSGO Ipameri grew to an average height of 25.3 cm while the susceptible BRSGO Luziânia grew to 22.2 cm. The resistant cultivar had no symptoms of nematode infestation.

Results showed differences between the two cultivars for all the variables evaluated either in the field or greenhouse experiments. There was interaction between cultivars and doses of abamectin for the nematode population, and a quadratic effect was observed on the susceptible cultivar (Fig. 1). Interaction between doses of abamectin and thiabendazole was also observed for the number of females and eggs per female on the susceptible cultivar BRSGO Luziânia (Fig. 2). Seed treatments did not affect the number of females on roots or the number of cysts in soil on either the resistant or the susceptible cultivar in the greenhouse.

*Heterodera glycines* population measured as number of females on the roots, number of eggs per female, and number of cysts/100 cm<sup>3</sup> of soil was higher on the susceptible cultivar BRSGO Luziânia both in the field (Table 1) and greenhouse (Table 2) confirming the resistance and susceptibility of the cultivars in relation to *H. glycines* race 3 (Dias *et al.*, 2005). *Heterodera glycines* reproduced on the resistant cultivar to some extent, although at a much lower rate, than in the susceptible cultivar across all treatments. Asmus *et al.* (2001) reported no difference in penetration of either the susceptible or the resistant cultivar by J2 of *H. glycines*. However, the reproductive development of *H. glycines* on resistant cultivars was limited. Li *et al.* (2004) demonstrated that the presence of the gene rhg 1, derived from PI 88788, which confers resistance to race 3, does not inhibit nematode penetration but suppresses its growth, development, and female fertility.

Under both field and greenhouse conditions, no significant effect of the nematicide seed treatments on the *H. glycines* population density was observed on the resistant cultivar BRSGO Ipameri. This is likely due to the very low number of females and cysts that were found on this cultivar.

The effect of seed treatment with abamectin on *H. glycines* population was observed in the susceptible cultivar BRSGO Luziânia in the field experiment (Fig. 1). There was a quadratic relationship between the number of females on roots and increasing doses of abamectin ( $y = -0.0605x^2 + 2.5412x + 73.523$ ,  $R = 0.99$ ), cysts in the soil ( $y = -0.0309x^2 + 0.8182x + 74.903$ ,  $R = 0.99$ ) and eggs per female ( $y = -0.0324x^2 + 0.7418x + 119.42$ ,  $R = 0.87$ ) with a trend toward

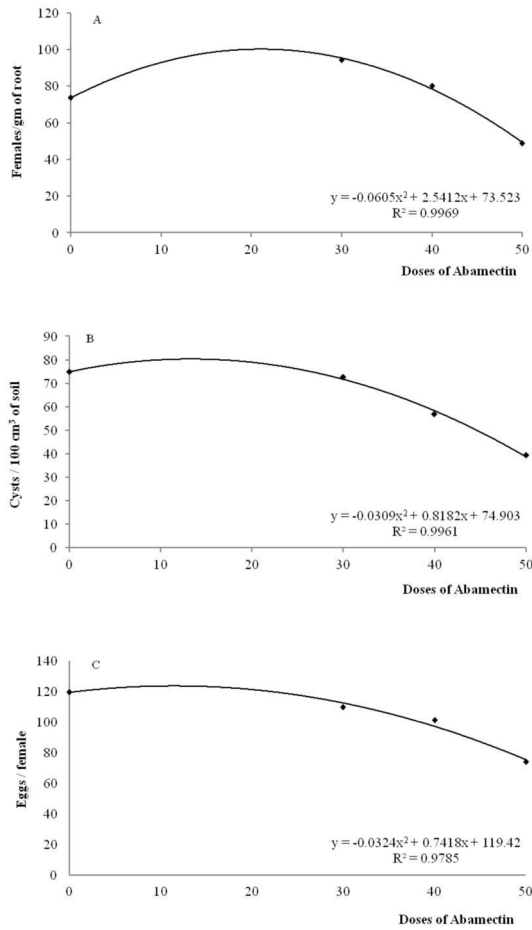


Fig. 1. Population density of *Heterodera glycines* under different abamectin rates on the susceptible soybean cultivar (BRSGO Luziânia). A-Females on roots; B-cysts in the soil, C-eggs per female (field experiment).

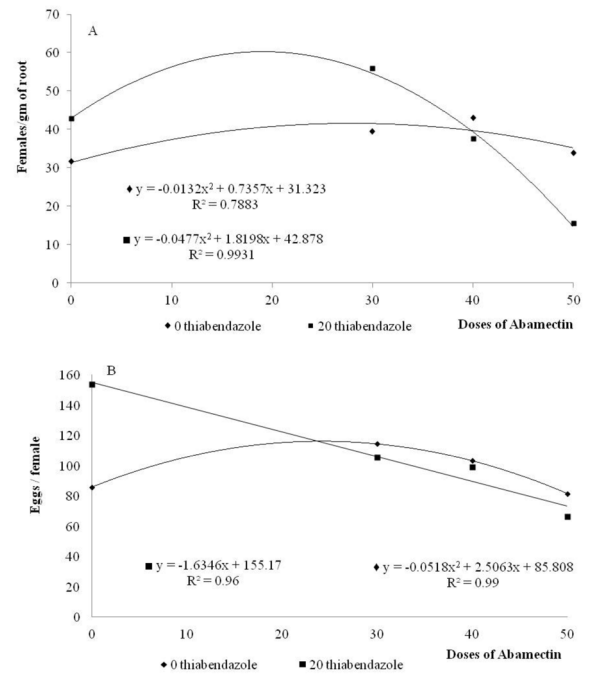


Fig. 2. Population density of *Heterodera glycines* under the effect of abamectin rates, with or without thiabendazole, on the susceptible soybean cultivar BRSGO Luziânia. A-females on roots, B-eggs per female (field experiment).

Table 1. Effect of soybean cultivars on the numbers of *Heterodera glycines* females in roots, eggs/female, and cysts/100 cm<sup>3</sup> of soil at 30, 45, and 60 days after sowing (DAS), in field experiment conducted in 2006 and 2007.

	30 DAS		45 DAS		60 DAS	
	BRSGO Ipameri	BRSGO Luziânia	BRSGO Ipameri	BRSGO Luziânia	BRSGO Ipameri	BRSGO Luziânia
Females/root <sup>y</sup>	1.4 a <sup>x</sup>	135.2 b	0.1 a	36.8 b	0.4 a	50.5 b
CV (%)	9.7		6.0		6.6	
Eggs/female <sup>y</sup>	33.5 a	202.5 b	5.2 a	195.4 b	15.4 a	155.0 b
CV (%)	14.3		7.7		8.1	
Cysts/100cm <sup>3z</sup>	1.3 a	82.5 b	1.1 a	58.2 b	2.4 a	63.6 b
CV (%)	51.9		43.3		32.9	

<sup>x</sup>Means followed by the same letter in the row do not differ from each other (F-test,0.05). Comparisons between cultivars within each evaluation timing.

<sup>y</sup>Analysis based on  $x^{-0.1}$  transformed data

<sup>z</sup>Analysis based on  $(x + 0.1)^{0.2}$  transformed data.

Table 2. Mean number of female *Heterodera glycines* on roots and cysts in the soil in resistant (BRSGO Ipameri) and susceptible (BRSGO Luziânia) soybean cultivars at 30 d after inoculation (DAI), as a function of seed treatments with abamectin and thiabendazole (greenhouse experiment).

Abamectin g ha <sup>-1</sup>	Thiabendazole g ha <sup>-1</sup>	Females/two plants <sup>y</sup>		Cysts/100 cm <sup>3</sup> of soil <sup>y</sup>	
		BRSGO Ipameri	BRSGO Luziânia	BRSGO Ipameri	BRSGO Luziânia
0	0	3.0 a <sup>x</sup>	13.6 a	1.2 a	35.4 a
0	20	2.0 a	14.5 a	2.0 a	27.8 a
30	0	1.8 a	17.4 a	0.8 a	39.6 a
30	20	2.1 a	15.8 a	3.0 a	13.2 a
40	0	2.1 a	14.3 a	0.4 a	31.0 a
40	20	1.5 a	17.3 a	0.0 a	20.8 a
50	0	1.9 a	17.8 a	0.4 a	24.8 a
50	20	1.7 a	16.8 a	0.2 a	24.2 a
Mean		2.0A	15.9B	1.0A	27.1B
CV (%)		29.96		91.66	

<sup>x</sup>Means followed by the same letter do not differ by the F test, at 5%.

<sup>y</sup>Analysis were based on square root transformed data  $(x + 0.1)$ .

population reduction at the higher rates, confirming the results of Monfort *et al.* (2006). Since the residual period of the product is approximately 45 d, the same or a higher nematicidal effect would be expected in the early stages of crop development as observed by Faske and Starr (2007) in abamectin-treated cotton seeds to control *M. incognita* and *R. reniformis*.

As reported by Penteadó *et al.* (2005) for the pathosystem cotton × *M. incognita*, protection of plant roots in the early crop stages is desirable because it can improve the crop performance and yield. In our study, however, the beneficial effect of abamectin seed treatment did not increase yield, and only a cultivar effect was observed for this variable. The yield of cultivar BRSGO Ipameri was 2,213 kg/ha and BRSGO Luziania yielded 679 kg/ha.

There was a significant interaction between rates of thiabendazole and abamectin ( $P < 0.05$ ) for the number of females on roots and number of eggs per female (Fig. 2). Without the use of thiabendazole, the number of females on roots (Fig. 2a) and eggs/female (Fig. 2b) did not show important variation as the abamectin doses were increased. However, when thiabendazole was added to the seed treatment, the nematode population was higher at low doses or without abamectin. The number of females reduced at the highest abamectin rate (Fig. 2a) and the number of eggs/female decreased linearly as abamectin rates were increased (Fig. 2b). Thus, these results indicate that the combined use of thiabendazole and abamectin should only be used at abamectin rates above 40 g ha<sup>-1</sup>.

Resistant cultivar BRSGO Ipameri supported less nematode reproduction and had higher yield than the susceptible BRSGO Luziania. Crop yield was not affected by the seed treatment.

Seed treatment with abamectin had little effect on *H. glycines* populations on the susceptible soybean cultivar and none on a resistant cultivar.

Association of thiabendazole with abamectin for soybean seed treatment should not be used at abamectin rates lower than 40 g a.i. 100 kg<sup>-1</sup> seeds.

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