VIRULENCE OF ENTOMOPATHOGENIC NEMATODES (RHABDITIDA: STEINERNEMATIDAE AND HETERORHABDITIDAE) FOR THE CONTROL OF *Diabrotica speciosa* GERMAR (COLEOPTERA: CHRYSOMELIDAE)

Virulência de nematoides entomopatogênicos (Rhabditida: Steinernematidae e Heterorhabditidae) para o controle de *Diabrotica speciosa* Germar (Coleoptera: Chrysomelidae)

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ABSTRACT

Entomopathogenic nematodes (EPNs) are used in biological control of soil insects and show promise in the control of *D. speciosa*. The objective of this work was to evaluate the potential of native and exotic entomopathogenic nematode isolates in the control of *D. speciosa* under laboratory and greenhouse conditions. Results showed that all of EPNs caused larval mortality. The most virulent were *Heterorhabditis* sp. RSC01 (94%), *Steinernema glaseri* (84%), *Heterorhabditis* sp. JPM04 (82%) and *Heterorhabditis amazonensis* RSC05 (78%). There was no effect of the *Heterorhabditis* sp. RSC01 and *S. glaseri* isolates on eggs. The maximum mortality of *D. speciosa* larvae by *Heterorhabditis* sp. RSC01 was observed at a concentration of 300 IJ/ insect, while by *S. glaseri* observed the highest mortality at the concentration of 200 IJ/ insect. The *Heterorhabditis* sp. RSC01 isolate caused over 80% pupal mortality at a concentration of 250 IJ/insect. The virulence of *Heterorhabditis* sp. RSC01 and *S. glaseri* was affected by temperature. The *Heterorhabditis* sp. RSC01 isolate caused reduction in larva survival under greenhouse conditions at all of the tested concentrations and there was no difference in mortality among different concentrations of infectid juveniles.

Index terms: *Steinernema*, *Heterorhabditis*, crisomelid beetle, pathogenicity.

RESUMO

Os nematóides entomopatogênicos são utilizados no controle biológico de pragas de solo, e são promissores para o controle de *D. speciosa*. Neste trabalho, objetivou-se avaliar o potencial de espécies nativas e exóticas de isolados de nematóides entomopatogênicos para o controle de *D. speciosa*, em condições de laboratório e de casa de vegetação. Verificou-se que todos os nematóides causaram mortalidade larval. Os mais virulentos foram *Heterorhabditis* sp. RSC01 (94%), *Steinernema glaseri* (84%), *Heterorhabditis* sp. JPM04 (82%) e *Heterorhabditis amazonensis* RSC05 (78%). Não houve efeito dos isolados *Heterorhabditis* sp. RSC01 e *S. glaseri* em ovos. A mortalidade máxima de larvas de *D. speciosa* por *Heterorhabditis* sp. RSC01 foi observada na concentração de 300 JI / inseto, enquanto para *S. glaseri* a maior mortalidade foi obervada na concentração de 200 JI/ inseto. O isolado *Heterorhabditis* sp. RSC01 causou mais de 80% de mortalidade de pupas na concentração de 250 IJ/ inseto. A virulência de *Heterorhabditis* sp. RSC01 e *S. glaseri* foi afetada pela temperatura. O isolado *Heterorhabditis* sp. RSC01 causou redução na sobrevivência da larvas em casa de vegetação em todas as concentrações de juvenis infectantes testadas e não houve diferença na mortalidade entre os diferentes tratamentos.

Termos para indexação: *Steinernema*, *Heterorhabditis*, crisomelídeo, patogenicidade.

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INTRODUCTION

The chrysomelid *Diabrotica speciosa* Germar (Coleoptera: Chrysomelidae) has been causing great concern to farmers, due to its wide occurrence and polyphagous habit. The adults feed preferentially on leaves, shoots, fruits and pollen of cultivated and wild plants, while the larvae prefer the roots. In early attacks on maize, the larvae of *D. speciosa* can bore into the caulicle of the seedlings, causing drying and death of central leaves. In more developed plants however, they prefer to feed on adventitious roots (GASSEN, 1989). There are few works looking at the use of biological control agents to suppress populations of *D. speciosa* at this stage; so control has is done exclusively through the use of chemical products.

Entomopathogenic nematodes (EPNs) of the Steinernematidae and Heterorhabditidae families (Rhabditida) are promising biological control agents for the control of soil pests (GREWAL et al., 2001). Studies using EPNs have been carried out in some countries with other species of the genus *Diabrotica* and have presented good results under both laboratory and field conditons

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(JACKSON; BROOKS, 1989; WRIGHT et al., 1993; JOURNEY; OSTLIE, 2000; KURTZ et al., 2007).

The objective of this work was to evaluate the virulence of native and exotic entomopathogenic nematode isolates against of *D. speciosa* under laboratory and greenhouse conditions.

MATERIAL AND METHODS

The research was conducted at the Insect Pathology Laboratory and greenhouse of the Entomology Department (END) of the Federal University of Lavras (UFLA), located in the county of Lavras, MG, from December, 2007 to December, 2008.

Rearing of *Diabrotica speciosa* and multiplication of entomopathogenic nematodes

For the maintenance of adults and the obtaining of eggs and larvae of *D. speciosa*, the modified methodology described by Ávila and Milanez (2004) was used. Adults of *D. speciosa* were collected from bean crops in an experimental field of UFLA and from the Palmital Farm, in the municipal district of Ijaci, MG.

Adults were kept in cylindrical glass jars 17cm high and 10cm in diameter, with a perforated plastic cover, in an acclimatized chamber (temperature 25±1º C, humidity 70±10% and 14 hour photophase).

The jars were lined with a disk of paper towel to avoid excess humidity. Bean leaves were placed within the jars to feed the insects, as well as a 5 cm diameter Petri dish containing a piece of black colored moistened gauze, to provide a surface for oviposition. On the surface of the gauze seminal rootlets of corn were placed to stimulate the oviposition of the females.

The eggs were removed from the oviposition substrate, by washing the gauze in running water over a fine fabric (voile), where they were retained. To avoid the contamination by fungi during the incubation period, the eggs were treated with a 1% copper sulfate (CuSO4) solution for two minutes and, soon afterwards, transferred to 9 cm diameter Petri dishes lined with humid filter paper, which were maintained in an acclimatized chamber (25±1º C, 70±10% humidity and 14 hour photophase) until the larvae hatched.

The larvae were maintained in plastic pots containing vermiculite and corn seedlings according to the methodology described by Ávila et al. (2000) until the beginning of the experiments.

Multiplication of EPNs was conducted using five larvae of *Galleria mellonella* Linnaeus (Lepidoptera: Pyralidae) raised in the laboratory, according to the methodology described by Dutky et al. (1964) and fed with a modified artificial diet (C. Dolinski, personal communication, March, 2008). The nematodes were multiplied according to the methodology described by Molina and López (2001).

Virulence test of entomopathogenic nematodes to third instar larvae of *Diabrotica speciosa*

The virulence against *D. speciosa* larvae of the seventeen isolates of EPNs, belonging to the genera *Heterorhabditis* and *Steinernema* was evaluated. The bioassay consisted of five repetitions, each made up of a 9 cm Petri dish containing 15 g of vermiculite, 1.5 g of corn roots and 9 mL of water, to which were transferred ten third instar *D. speciosa* larvae.

The experimental design was entirely random, with eighteen treatments (17 EPNs isolates and the control). 1 mL of aqueous suspension of EPNs was added at a concentration of 1500 infective juveniles (IJ)/mL, corresponding to 150 IJ/insect, while 1 mL of distilled water was added to the control. The Petri dishes were maintained under controlled conditions at 25±1º C, 70±10% humidity and with a 14 hour photophase.

The evaluations were conducted three days after the application of the nematodes, by counting the number of dead larvae. The symptomatology of the dead larvae was verified by the confirmation of the *causa mortis*, according to the typical characteristics of death caused by nematodes. The larvae killed by nematodes of the genus *Heterorhabditis* presented a red color, while for *Steinernema* spp. they were of a whitish color.

The mortality data was submitted to the variance analysis and compared, by the Scott-Knott test of averages, to 5% probability using the SISVAR (FERREIRA, 2002) statistical program.

Test with eggs

The bioassay consisted of five repetitions, each made up of a 5 cm diameter Petri dish lined with a sheet of filter paper, to which ten five-day-old eggs of *D. speciosa* were transferred.

The experimental design was fully randomized. An aqueous suspension of 0.3 mL of the EPN isolates *Heterorhabditis* sp. RSC01 and *S. glaseri* were added to the Petri dish, at concentrations of 25, 50, 100, 150, 200 and 250 IJ/insect. 0.3 mL of distilled water was added to the control and every other day, 0.25 mL of distilled water was added to each of the Petri dishes, to maintain humidity levels. The Petri dishes were maintained under controlled conditions at a temperature of 25±1º C, 70±10% humidity and with a 14 hour photophase. Daily evaluations were made by counting the larvae that had emerged from the eggs in each repetition.

The data on egg mortality, obtained at different concentrations were submitted to the regression analysis at 5% of probability, using the SISVAR statistical program, with the mortality of the insect as the dependent variable and the concentrations of the nematode as the independent variable.

Test with third instar larvae

The test consisted of five replicates, each made up of a 9 cm diameter Petri dish, containing 15 g of vermiculite, 1.5 g of corn roots and 9 mL of distilled water, to which ten third instar *D. speciosa* larvae were transferred. The experimental design was fully randomised. 1 mL of aqueous suspension of EPN *Heterorhabditis* sp. isolate RSC01 and *S. glaseri* were added at the concentrations of 50, 100, 150, 200, 250 and 300 IJ per insect and, 1mL of distilled water was added to the control. The Petri dishes were maintained under controlled conditions at 25±1º C, 70±10% humidity and with a 14 hour photophase. The evaluations were made three days after the start of the experiment, by counting the dead larvae in each repetition.

Test with pupae

The bioassay consisted of four repetitions, each made up of a 9 cm diameter Petri dish containing 15 g of vermiculite and 9 mL of distilled water, to which eight *D. speciosa* pupae were transferred. The experimental design was fully randomized. 1 mL of aqueous suspension of EPNs/ Petri dish was added at the concentrations of 100, 150, 200, 250 and 300 IJ/insect of the *Heterorhabditis* sp. RSC01 isolates. 1mL of distilled water/Petri dish was added to the control. The Petri dishes were maintained under controlled conditions at a temperature of 25±1º C, 70±10% humidity and with a 14 hour photophase. The evaluations were conducted three days after the start of the experiment, by counting the pupae killed by nematodes in each repetition. The pupae mortality caused by nematodes was confirmed by observing the symptomatology, since the pupae killed by *Heterorhabditis* sp. RSC01 presented a red coloration.

Effect of temperature on the mortality of *Diabrotica speciosa* larvae by entomopathogenic nematodes

A bioassay was conducted to study the effect of temperature on the infectivity of IJ, using the *Heterorhabditis* sp. RSC01 and *S. glaseri* nematodes. The bioassays consisted of five repetitions/ treatment, each made up of a 9 cm diameter Petri dish containing 15 g of sterilized vermiculite, 1.5g of corn roots and 9 mL of distilled water, to which eight third instar *D. speciosa* larvae were transferred. The treatments were run at the constant temperatures of 15, 20, 22, 25 and 28º C, in five acclimatized chambers, in which the Petri dishes remained for three days, with 70±10% RH and a 12 hour photophase. 1 mL of standardized aqueous suspension was added at the concentration of 200 IJ/insect, which was the concentration that caused the highest mortality of *D. speciosa* larvae, according to tests conducted under laboratory conditions. The experimental design was entirely random.

Evaluations were made three days after the start of the experiments, by counting the larvae killed EPNs in each repetition.

Mortality data (larvae and pupae) at different tested concentrations of the infective juveniles or different temperatures were compared using generalized linear models (GLM) with logit link function and binary distribution, using the Statistical Analysis System (SAS) (SAS INSTITUTE, 2003).

Susceptibility of *D. speciosa* larvae to entomopathogenic nematodes under greenhouse conditions

The bioassay consisted of six repetitions, each made up of a plastic vase with 2.5 liter capacity, containing sterilized soil and sterilized vermiculite medium (1/10) and 500 mL of distilled water. Eight corn seeds were sowed in each vase, which were thinned after seven days, leaving only five plants per vase. Twenty third instar *D. speciosa* larvae were transferred to each vase.

The larvae were placed at a depth of five centimeters below the soil level, close to the plant roots. Twenty-four hours after the larvae were transferred, 50 mL of EPNs aqueous suspension were added, at the concentrations of 6,500, 13,000, 26,000 and 52,000 IJ of *Heterorhabditis* sp. RSC01, per repetition (vase). The vases corresponding to the control received 50 mL of distilled water. The concentration of 6,500 IJ/vase was established according to laboratory test results that determined the maxim lethal concentration of *Heterorhabditis* sp. RSC01 on third instar *D. speciosa* larvae. The experimental design was entirely random.

The average temperature registered during the experiment was 27.4º C and the average relative humidity 62%. The evaluations were conducted five days after the start of the experiment, by counting the live larvae per each repetition. The survival data was submitted to the variance analysis and the averages were compared by the Tukey test at 5% probability, using the SISVAR statistical program.

RESULTS AND DISCUSSION

Virulence test

All of the tested nematodes caused mortality to third instar *D. speciosa* larvae, differing from the control, in which mortality was not observed. However, the virulence varied among the different isolates (Table 1).

Table 1 – Percentage mortality of third instar larvae of *Diabrotica speciosa* by different isolates of entomopathogenic nematodes at a concentration of 150 IJ / insect. (Temp.: 25 ± 1º C, RH: 70 ± 10% and photophase: 14 h).

Treatment (EPN isolates)	Place of Origin	Average Mortality ± EP (%)[1]
Heterorhabditis sp. RSC01	Amazonas/ Brasil	94±2.34 a
Steinernema glaseri CCA	Sao Paulo/ Brasil	84±3.66 a
Heterorhabditis sp. JPM04	Minas Gerais/ Brasil	82±2.89 a
Heterorhabditis amazonensis RSC05	Amazonas/ Brasil	78±3.61 a
Heterorhabditis sp. RSC02	Amazonas/ Brasil	74±4.68 b
Heterorhabditis bacteriophora	New Jersey/ USA	72±2.89 b
Heterorhabditis sp. JPM03	Minas Gerais/ Brasil	72±2.89 b
Heterohabditis sp. RSC03	Amazonas/ MG	70±3.76 b
Heterorhabditis sp. ALHO	Minas Gerais/ Brasil	68±3.61 b
Heterorhabditis sp. PI	Piaui/ Brasil	68±439 b
Steinernema feltiae SN	Florida/ USA	66±2.99 b
Steinernema carpocapsae A11	North Carolina/ USA	64±4.55 b
Heterorhabditis sp. SORGO	Minas Gerais/ Brasil	56±2.99 b
Steinernema anomali	Voronezh/ Russia	54±4.42 b
Heterorhabditis sp. JPM01	Minas Gerais/ Brasil	46±2.34 c
Heterorhabditis sp. HP88	New Jersey/ USA	38±4.66 c
Steinernema riobrave 355	Texas/ USA	30±3.98 c
Control		0±0 d

[1]Averages followed by same letter do not differ by Scott-Knott test ($p < 0.05$).

Among the seventeen isolates tested, fourteen caused over 50% mortality of *D. speciosa* larvae. The most virulent were the isolates *Heterorhabditis* sp. RSC01 (94%), *S. glaseri* (84%), *Heterorhabditis sp.* JPM04 (82%) and *Heterorhabditis amazonensis* RSC05 (78%) (Table 1).

These results are similar to those observed by Toepfer et al. (2005) who, while working with the isolate *S. glaseri* NC (USA origin), saw that this was pathogenic to third instar larvae of *Diabrotica virgifera virgifera* LeConte, 1868 (Coleoptera: Chrysomelidae) under laboratory conditions. Converse and Grewal (1998), seeking to select EPNs for the control of *Cyclocephala hirta* LeConte, 1861 (Coleoptera: Scarabaeidae), verified that isolate NJ65 of *S. glaseri* was the most virulent among the 22 isolates tested, causing 76.5% larval mortality under laboratory conditions, after three days of exposure to IJ.

It may be noticed that the most virulent nematodes are from Brazil (Table 1). In addition, native isolates have better chances of adaptation to the Brazilian environmental conditions, as well as to the local insect fauna (DOLINSKI; MOINO Jr., 2006).

In this research, several native isolates were used and they showed potential for the control of *D. speciosa* larvae. These should also be investigated regarding their specificity, to commercially exploit only those that present a high selectivity to the natural enemies (DOLINSKI; MOINO JR., 2006).

Test with eggs

There was no effect of the nematodes *Heterorhabditis* sp. RSC01 and *S. glaseri* on the mortality of *D. speciosa* eggs at any of the tested concentrations.

These results are similar to those related by Jackson and Brooks (1995) who, working with *D. v. virgifera*, did not verify an effect of the nematode *S. carpocapsae* on the mortality of eggs of this insect, under laboratory conditions. Journey and Ostlie (2000), testing *S. carpocapsae* for the control of *D. v. virgifera* in the field, also verified that this isolate did not present an egg viability reduction.

According to Jackson and Brooks (1995), the absence of infectivity of EPNs in crisomelid beetle eggs can be related to the impermeability of the eggs of those organisms. Toepfer and Kuhlmann (2004) related the natural occurrence of native EPNs in *D. v. virgifera* eggs in Central Europe, however, the identification of the isolates was not conducted.

Those results differ from those observed by Machado et al. (2005) who, working with *Mygdolus fryanus* Westwood, 1863 (Coleoptera: Vesperidae) verified 53.33% egg mortality, inoculating 60 IJ/egg of *Heterorhabditis indica* Poinar, Karunakar and David 1992, not differing from the concentration of 600 IJ/egg (60%).

Tests with third instar larvae

The mortality of larvae caused by *Heterorhabditis* sp. RSC01 varied between 64 and 96% (Table 2), at the concentrations of 50 and 300 IJ/insect and between 34 and 90%, at the concentrations of 50 and 200 IJ/insect, respectively, when applying *S. glaseri*.

Table 2 – Percentage of confirmed mean mortality of larvae of *Diabrotica speciosa* at different concentrations of infective juveniles of *Heterorhabditis* sp. RSC01 and *Steinernema glaseri* (temperature 25 ± 1º C, RH 70 ± 10% and of 14 hour photophase).

Treatment (JI/ larvae)	Mean mortality (%) ± Standard Error Mean[1]	
	Heterorhabditis sp. RSC01	*Steinernema glaseri*
0	0 d[1]	2 ± 2 c
50	64 ± 6.8 c	34 ± 6.7 b
100	82 ± 5.4 bc	70 ± 6.5 ab
150	86 ± 4.9 ab	74 ± 6.2 ab
200	92 ± 3.8 ab	90 ± 4.2 a
250	88 ± 4.6 ab	80 ± 5.7 a
300	96 ± 2.8 a	88 ± 4.6 a

[1]Means followed by the same letter do not differ at 5% probability when compared to generalized linear models.

The maximum mortality of *D. speciosa* larvae by *Heterorhabditis* sp. RSC01 was observed at a concentration of 300 IJ/ insect, corresponding to 47.16 IJ/ cm^2. In the bioassay of *S. glaseri* the highest mortality was observed at the concentration of 200 IJ/ insect (31.44/ cm^2).

Toepfer et al. (2005), testing the virulence of *S. glaseri* on third instar larvae of *D. v. virgifera* at different concentrations, verified that there was an increase in the mortality of larvae with the increase of the concentration, observing a mortality superior to 77% at the concentration of 15.9 IJ/ cm^2. These results are similar to those observed by Kurtz et al. (2009) who reported that when testing *H. bacteriophora*, *H. megidis* and *S. feltiae* at a concentration of 16 IJ/ cm^2 in trays with sand, they observed the maximum mortality of the third instar larvae (87.8%) in the treatment with *H. bacteriophora*.

Tests with pupae

The *Heterorhabditis* sp. RSC01 isolate was pathogenic to pupae of *D. speciosa*, causing mortality at all of the studied concentrations (Table 3). Concentrations of 200, 250 and 300 IJ/ insect did not differ among themselves and caused high mortality of *D. speciosa* pupae.

Table 3 – Percentage of confirmed mean mortality of pupae of *Diabrotica speciosa* at different concentrations of infective juveniles of *Heterorhabditis* sp. RSC01 isolate (25 ± 1º C, RH 70 ± 10% and a photophase of 14 hours).

Treatment (JI/ pupae)	Mean mortality (%) ± Standard Error Mean [1]
0	18.75 ± 6.9 b[1]
100	75 ± 7.6 ab
150	68.75 ± 8.1 ab
200	7.50 ± 5.8 a
250	93.75 ± 4.3 a
300	93.75 ± 4.3 a

[1]Means followed by the same letter do not differ at 5% probability when compared to generalized linear models.

Yang et al. (2003), working with *S. feltiae* for the control *Luperomorpha suturalis* Chen, 1938 (Coleoptera: Chrysomelidae), verified pupae mortality of 97.1 and 83%, under laboratory conditions, at a temperatures of 25 and 15º C, respectively.

The effect of the nematodes on the *D. speciosa* pupae can increase the long term action of these entomopathogens in the reduction of field populations of this insect, interfering in population growth and, consequently, avoiding re-infestations by the pest. Furthermore, the reduction of the adult population in the field can contribute to the reduction of the damage that these insects cause to the aerial part of the plants.

Effect of Temperature on the Mortality of Larvae of *D. speciosa* by Entomopathogenic Nematodes

At 20º C we observed a low mortality of larvae in both treatments (*S. glaseri* and *Heterorhabditis* sp. RSC01) (Table 4). To *Heterorhabditis* sp. RSC01 at the temperatures of 25 and 28° C we verified high mortality of larvae, and no mortality was observed at 15° C.

The bioassay of *S. glaseri*, at temperatures of 22 and 25° C caused high mortality of larvae, whereas at 28° C there was a reduction in mortality, suggesting that this nematode may be more sensitive to high temperatures.

These results are in accordance with those observed by Boivin and Belair (1989) who, working with *S. feltiae* for the control of *Listronatus oregonensis* LeConte, 1857 (Coleoptera: Curculionidae), verified a decrease in TL_{50} with the increase of the temperature.

Table 4 – Percentage of confirmed mean mortality of larvae of *Diabrotica speciosa* by *Heterorhabditis* sp. RSC01 and *Steinernema glaseri* isolates at different temperatures (RH 70 ± 10% and of 14 hour photophase).

Treatment (°C)	Mean mortality (%) ± Standard Error Mean	
	Heterorhabditis sp. RSC01	*Steinernema glaseri*
15	0 c[1]	5 ± 3.4 c
20	17.50 ± 6 b	25 ± 6.8 bc
22	60 ± 7.7 ab	58.33 ± 10.06 a
25	82.50 ± 6 a	62.50 ± 7.6 a
28	85 ± 5.6 a	47.50 ± 7.9 ab

[1]Means followed by the same letter do not differ at 5% probability when compared to generalized linear models.

Temperature is the main factor that interferes in the mobility of the nematodes and, consequently, in the expense of their nutritional reserves and in the survival of IJ (MOLYNEUX, 1985). The reduction in the mortality of *D. speciosa* larvae at the lowest temperatures, for both isolates tested, is a factor that should be taken into account when employing this entomopathogen in the field, keeping in mind its wide territorial distribution and the different climatic conditions in the areas in which this insect is found. In colder environments, insect mortality over a short period of time could be affected.

Susceptibility of *D. speciosa* Larvae to Entomopathogenic Nematodes under Greenhouse conditions

The *Heterorhabditis* sp. RSC01 nematode reduced the survival of *D. speciosa* larvae in the soil at all of the tested concentrations, when compared to the control, without, however, differing among themselves (Figure 1).

The non-verification of mortality differences of *D. speciosa* larvae among the four tested concentrations demonstrates evidence that the concentration of 6,500 IJ was sufficient to cause the maximum mortality in the greenhouse, the use of higher concentrations being unnecessary under this condition.

These results are similar to those observed by Riga et al. (2001). Testing the efficiency of the *S. glaseri* and *S. feltiae* nematodes against four species of corn pests, among them crisomelid beetle larvae, under laboratory and greenhouse conditions, these authors verified that both tested species presented an effect on the mortality of all of the pest species, resulting in lower damage incidence in the corn plants in the parcels that received the EPN treatments in relation to the control.

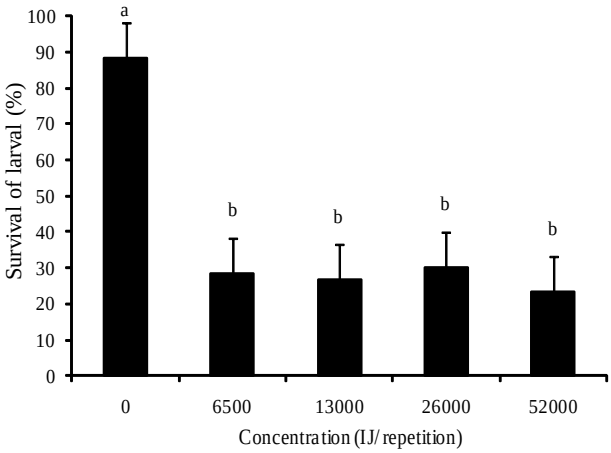

Figure 1 – Average percentage survival of *Diabrotica speciosa* larvae at different concentrations of infective juveniles of *Heterorhabditis* sp. RSC01 per pot, under greenhouse conditions.

The concentration of *Heterorhabditis* sp. RSC01 that caused the maximum mortality of *D. speciosa* larvae under laboratory and greenhouse conditions (31.41 IJ/cm^2, equivalent to 3.14 x 10^9 IJ/ha) is similar to the concentration used by Yang et al. (2003) that studied *S. feltiae* for the control of *L. suturalis* in the field and verified 77.8% larval reduction at the concentration of 30 IJ/cm^2, thirty-eight days after the treatment and 92.4% larval reduction a hundred days after the treatment. Wright et al. (1993), working with *S. carpocapsae* applied in the field for the control of *D. v. virgifera*, under center pivot irrigation in the corn culture, observed reduction in the damage rate of the plants. They also noticed a reduction in adult emergence, with nematode application at concentrations of 1.2 and 2.5 x 10^9 IJ/ha, lower that mentioned in this research. Likewise, Thurston and Yule (1990), testing the nematodes *S. feltiae* All and *S. bibionis* Sn against *D. barberi* larvae in the field, in corn culture, observed that both species of EPNs tested reduced the number of larvae in the soil compared to the control, at concentrations of 1.3 x 10^8

and 1.3 x 10^9 IJ/ha, not verifying a difference among the species or the tested concentrations.

Toepfer et al. (2008), working with EPNs for the control of *D. v. virgifera* larvae under field conditions in the corn culture, verified that all of the species of nematodes (*H. bacteriophora, S. feltiae* and *H. megidis*) tested caused reduction of the insect population in the soil. However, the most virulent species was *H. bacteriophora*, that caused an 81% reduction. The same authors also verified that the tested species reduced the percentage of damage to the plants, proving the potential of EPNs for the control of crisomelid beetle larvae in corn culture.

Kurtz et al. (2007) also demonstrated that the *H. bacteriophora, H. megidis* and *S. feltiae* species present a capacity to establish themselves in corn for a period of up to five months, being promising for the control of *D. v. virgifera* in this culture, with massive applications of those EPNs in the field.

CONCLUSION

The high potential that EPNs demonstrate in laboratory, greenhouse and field studies in the control of crisomelid beetle larvae, as well as the results obtained in this study from laboratory and greenhouse tests, confirms the great potential of *Heterorhabditis* sp. RSC01 for the control of *D. speciosa* larvae. Their use can be viable in field studies seeking the control of this pest in such cultures as irrigated corn and, in particular potato as it is cultivated in smaller areas, requiring high moisture levels for its development, a characteristic that favors the survival and behavior of EPNs.

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REFERENCES

ÁVILA, C.J.; MILANEZ, J.M. Larva-alfinete. In: SALVADORI, J.R.; ÁVILA, C.J.; SILVA, M.T.B. **Pragas de solo no Brasil.** Passo Fundo: Embrapa Trigo, 2004, p.211-232.

ÁVILA, C.J.; TABAI, A.C.P.; PARRA, J.R.P. Comparação de técnicas para a criação de *Diabrotica speciosa* (Germar) (Coleoptera: Chrysomelidae) em dietas natural e artificial. **Anais da Sociedade Entomológica do Brasil,** Londrina, v.29, n.2, p.257-267, jun. 2000.

BOIVIN, G.; BELAIR, G. Infectivity of two strains of *Steinernema feltiae* (Rhabditida: Steinernematidae) in relation to temperature, age, and sex of carrot weevil (Coleoptera: Curculionidae) adults. **Annals of the Entomological Society of America,** Lanham, v.82, n.3, p.762-765, jun. 1989.

CONVERSE, V.; GREWAL, P.S. Virulence of entomopathogenic nematodes to the western masked chafer *Cyclocephala hirta* (Coleoptera: Scarabaeidae). **Journal of Economic Entomology,** Lanham, v.91, n.2, p.428-432, apr. 1998.

DOLINSKI, C.; MOINO JÚNIOR, A. Utilização de nematóides entomopatogênicos nativos ou exóticos: o perigo das introduções. **Nematologia Brasileira,** Brasília, v.30, n.2, p.139-149, 2006.

DUTKY, S.R.; THOMPSON, J.V.; CANTWELL, G.E. A technique for the mass propagation of the DD-136 nematode. **Journal of Insect Pathology,** San Diego, v.6, n.4, p.417-422, 1964.

FERREIRA, D. F. **SISVAR Sistemas de Análises de Variância para dados balanceados:** programa de análises estatísticas e planejamento de experimentos. Versão 4.3. Lavras: UFLA, 2002.

GASSEN, D.N. **Insetos subterrâneos prejudiciais às culturas no sul do Brasil**. Passo Fundo: EMBRAPA-CNPT, 1989, 49 p. (Documentos, 13).

GREWAL, P.S.; NARDO, E.A.B.; AGUILLERA, M.M. Entomopathogenic nematodes: potential for exploration and use in South America. **Neotropical Entomology,** Londrina, v.30, n.2, p.191-205, jun. 2001.

JACKSON, J.J.; BROOKS, M.A. Susceptibility and immune response of western corn rootworm larvae (Coleoptera: Chrysomelidae) to the entomogenous nematode, *Steinernema feltiae* (Rhabditida: Steinernematidae). **Journal of Economic Entomology,** Lanham, v.82, n.4, p.1073–1077, aug. 1989.

JACKSON, J.J.; BROOKS, M.A. Parasitism of western corn rootworm larvae and pupae by *Steinernema carpocapsae*. **Journal of Nematology,** Lakeland, v.27, n.1, p.15-20, mar. 1995.

JOURNEY, A.M.; OSTLIE, K.R. Biological control of the western corn rootworm (Coleoptera:Chrysomelidae) using the entomopathogenic nematode, *Steinernema carpocapsae*. **Environmental Entomology**, Lanham, v. 29, n.4, p.822-831, aug. 2000.

KURTZ, B. et al. Assessment of establishment and persistence of entomopathogenic nematodes for biological control of western corn rootworm. **Journal of Applied Entomology,** Berlin, v.131, n.6, p.420-425, jul. 2007.

KURTZ, B. et al. Comparative susceptibility of larval instars and pupae of the western corn rootworm to infection by three entomopathogenic nematodes. **BioControl,** Dordrecht, v.54, n. 2, p. 255-262, apr. 2009.

MACHADO, L.A. et al. Patogenicidade de nematóides entomopatogênicos a ovos e larvas de *Migdolus fryanus* (Westwood, 1863) (Coleoptera: Vesperidae). **Arquivos do Instituto Biológico,** São Paulo, v.72, n.2, p.221-226, abr./jun. 2005.

MOLINA, J.P.; LÓPEZ, N.J.C. Producción in vivo de tres entomonematodos com dos sistemas de infección em dos hospedantes. **Revista Colombiana de Entomología,** Bogotá, v.27, n.1-2, p.73-78, ene./jun, 2001.

MOLYNEUX, A.S. Survival of infective juveniles *Heterorhabditis* spp., and *Steinernema* spp. (Nematoda: Rhabditida) at various temperatures and their subsequent infectivity for insects. **Revue Nematology,** v.8, n.2, p.165-170, 1985.

RIGA, E.; WISTLECRAFT, J.; POTTER, J. Potential of controlling insect pests of corn using entomopathogenic nematodes. **Canadian Journal of Plant Science,** Ottawa, v.81, n.4, p.783-787, oct. 2001.

SAS INSTITUTE. **SAS/ Stat 9.23 Service Pack 2.** Cary, 2003. 965p.

THURSTON, G.S.; YULE, W.N. Control of larval northern corn rootworm (*Diabrotica barberi*) with two Steinernematid nematode species. **Journal of Nematology,** Lakeland, v.22, n.1, p.127-131, jan. 1990.

TOEPFER, S.; KUHLMANN, V. Survey for natural enemies of the invasive alien chrysomelid, *Diabrotica virgifera virgifera*, in Central Europe. **BioControl,** Dordrecht, v.49, n.5, p.385-395, aug. 2004.

TOEPFER, S. et al. Screening of entomopathogenic nematodes for virulence against the invasive western corn rootworm, *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae) in Europe. **Bulletin of Entomological Research,** London, v.95, n.5, p.473-482, oct. 2005.

TOEPFER, S. et al. Comparative assessment of the efficacy of entomopathogenic nematode species at reducing western corn rootworm larvae and root damage in maize. **Journal of Applied Entomology,** Berlin, v.132, n.5, p.337-348, jun. 2008.

WRIGHT, R.J. et al. Efficacy and persistence of *Steinernema carpocapsae* (Rhabditida: Steinernematidae) applied through a center-pivot irrigation system against larval corn rootworms (Coleoptera: Chrysomelidae). **Journal of Economic Entomology**, Lanham, v.86, n.5, p.1348-1354, oct. 1993.

YANG, X. et al. Evaluation of entomopathogenic nematodes for control of the beetle, *Luperomorpha suturalis* Chen (Col., Chrysomelidae). **Journal of Applied Entomology,** Berlin, v.127, n.7, p.377-382, aug. 2003.