Assessment of entomopathogenic nematodes in *Agrotis ipsilon* (Lepidoptera: Noctuidae) under laboratory and greenhouse conditions

Evaluación de nematodos entomopatógenos en *Agrotis ipsilon* (Lepidoptera: Noctuidae)
bajo condiciones de laboratorio e invernadero

ANDRÉIA DE OLIVEIRA GIANNASI[1,2], CAMILA ROQUE BRAMBILA[1,3], MARCELO ZART[1,4],
BRUNA APARECIDA GUIDE[5] and VIVIANE SANDRA ALVES[1,6]

Abstract: This work aims to evaluate the potential of Brazilian isolates of entomopathogenic nematodes through the selection of isolates for *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae). In order to analyze the susceptibility at different instars of the insect and different concentrations, to investigate the *in vivo* production of these isolates, and to perform greenhouse tests in the Laboratory of Entomologia e Controle Microbiano of Universidade Estadual do Norte do Paraná, Brazil. Selection tests were carried out with nine isolates, and those that caused greater percentages of insect mortality were used in concentration tests (0, 50, 100, 150, 200 Infective Juveniles (IJs)/cm²), with different instars of the insect (2nd, 3rd, 4th, 5th, 6th instars and pupa), in the *in vivo* production of *A. ipsilon* larvae, and in pathogenicity tests in a greenhouse. In the selection test, the isolate CH3 (*Steinernema* sp.) caused 100 % of mortality in *A. ipsilon*, and the isolates GL (*Heterorhabditis amazonensis*), IBCBn-02 (*S. carpocapsae*) and IBCB-n05 (*H. indica*) caused 90 % mortality. In the tests of different concentrations, the isolate IBCB-n02 had CL_{99} estimated at a concentration of 140 IJs/cm², and the isolate IBCB-n05 had CL_{99} estimated at a concentration of 190 IJs/cm². The most susceptible larval stages were the 3rd and 4th instars. In the *in vivo* test for production of *A. ipsilon* larvae, no difference was observed between the two isolates. In the greenhouse test, the isolate IBCB-n02 was the only one that differed from the control treatment.

Key words: Cutworm, corn, biological control.

Resumen: El objetivo de este trabajo fue evaluar el potencial de los nematodos entomopatógenos (NEPs) de los géneros *Steinernema* y *Heterorhabditis* en el control de *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae). Para esto, se realizaron ensayos de selección de aislamientos, se evaluó la susceptibilidad en diferentes instares del insecto, se analizaron las diferentes concentraciones de NEPs y la producción *in vivo* de estos aislados, y se realizaron pruebas en invernadero en el Laboratorio de Entomología y control biológico de la Universidade Estadual do Norte do Paraná, Brasil. En los ensayos de selección con nueve aislamientos, los que causan mayor porcentaje de mortalidad en los insectos, se utilizaron diferentes concentraciones (0, 50, 100, 150, 200 Juveniles Infectivos/cm²) sobre diferentes instares del insecto (2do, 3er, 4to, 5to, 6to instar y pupa), en la producción *in vivo* en larvas de *A. ipsilon* y en pruebas de patogenicidad en invernadero. En el ensayo de selección, el aislamiento CH3 (*Steinernema* sp.) causó el 100 % de la mortalidad en *A. ipsilon* mientras que los aislados GL (*Heterorhabditis amazonensis*), IBCB-n02 (*S. carpocapsae*) e IBCB-n05 (*H. indica*) causaron 90 % de mortalidad. En la prueba de concentraciones, el aislado IBCB-n02 presentó una CL_{99} de 140 IJs/cm² y el aislado IBCB-n05, una CL_{99} de 190 IJs/cm². Las larvas de 3er y 4to instares fueron más susceptibles. En la prueba *in vivo* para la producción de larvas de *A. ipsilon*, no se observó diferencia entre los dos aislamientos. En el ensayo en invernadero, el aislado IBCB-n02 fue el único que difirió del tratamiento de control.

Palabras clave: Larva de polilla, maíz, control biológico.

Introduction

The black cutworm *Agrotis ipsilon* (Hufnagel, 1767) (Lepidoptera: Noctuidae) is a polyphagous pest that feeds on nearly all varieties of vegetables and many important grains, attacking mainly Solanaceae, Cruciferae and Curcubitaceae; however, they can also attack other species of different plant families, such as corn and soybeans (Link and Pedrolo 1987; Fernandes *et al.* 2013). This pest is a nocturnal insect, and when the larvae are touched, they roll up, becoming thread-like in appearance. During the day, the insect remains buried in the ground, which hinders its viewing field and consequently its control (Bento *et al.* 2007). In corn crop, younger larvae damage the leaves, and from the 3rd and 4th instars, they cut the base of the stem, causing streaks on the leaves, and the appearance of cuts in the seedlings at the base of the stem, resulting in losses of up to 27 % (Salvadori 2011).

The control of the black cutworm becomes difficult due to the insect's habits. Nevertheless, studies have reported the use of allelochemicals to inhibit the growth of larval stages (Reese and Beck 1976). In relation to the biological control, the presence of parasitoids and predators in the field and the consequent reduction in the insect population was reported by Link and Costa (1984). The use of *Bacillus thuringiensis* in the laboratory (Menezes *et al.* 2010) and in the field, by means of transgenic plants (Kullik *et al.* 2011), as well as the use of *Baculovirus* (Prater *et al.* 2006) have also been reported.

Entomopathogenic nematodes (EPNs) are insect parasites, which insecticide activity is given by a symbiosis with bacteria (*Xenorhabdus* and *Photorhabdus*), leading the host to death. This is one of the reasons of the successful microbial control (Grewal *et al.* 2001; Lewis *et al.* 2006). Infective juveniles (IJs) invade the body of their

[1] Universidade Estadual do Norte do Paraná, Av. Portugal, n. 340, Centro, CEP 86300-000, Cornélio Procópio, Paraná, Brazil. [2] Agronomist. M. Sc., Universidade Estadual do Norte do Paraná, Cornélio Procópio, Paraná, Brazil, *deiauenp@gmail.com*. [3] Biologist, Universidade Estadual do Norte do Paraná, Cornélio Procópio, Paraná, Brazil, *camilabuenp@gmail.com*. [4] Doctor in Agronomy, Universidade Estadual do Norte do Paraná, Cornélio Procópio, Paraná, Brazil, *marcelo_zart@yahoo.com.br*. [5] Ph. D. Student in Agronomy, Universidade Estadual de Londrina, Rodovia Celso Garcia Cid, Pr 445 Km 380. Cx. Postal 10.011. Londrina. Brazil. 86.057-970. *bruhguide@gmail.com*. [6] Pos-Doctor in Sciences, Universidade Estadual do Norte do Paraná, Cornélio Procópio, Paraná, Brazil, *vivialves@uenp.edu.br*. Corresponding author: Viviane Sandra Alves. Pos-Doctor in Sciences, Universidade Estadual do Norte do Paraná, Av. Portugal, n. 340, Centro, CEP 86300-000, Cornélio Procópio, Paraná, Brazil, *vivialves@uenp.edu.br*.

hosts and release their bacteria at the hemolymph stage, causing fast death of the insect by septicemia. Afterwards, the EPNs feed digested tissues of the dead insect and of the bacteria and reproduce themselves in several generations. When the food is depleted, the IJs leave the host searching for new hosts (Poinar 1990; Ferraz 1998).

EPNs present great tolerance to variations in environmental conditions. They can be used in combination with other control techniques, including chemical control, and are more recommended for the control of insect pests that present cryptic habitats associated with soil, since the nematodes can move themselves searching for a host (Ferraz 1998; Toledo *et al.* 2010).

Studies on EPNs are recent in Brazil. In the last few years, some works developed in the laboratory have demonstrated the potential of these agents to control several pests (Machado *et al.* 2005; Alves *et al.* 2009; Alves *et al.* 2012; Bortoluzzi *et al.* 2013). For instance, Leite *et al.* (2005), Andaló *et al.* (2010), and Andaló *et al.* (2012) studied insects of the order Lepidoptera, but there are no studies with Brazilian isolates aimed the control of *A. ipsilon*.

Thus, this work aims to evaluate the potential of Brazilian isolates of entomopathogenic nematodes by means of selection of isolates for *A. ipsilon*; to analyze the susceptibility at different instars of the insect and different concentrations; to investigate the *in vivo* production of these isolates; and to perform greenhouse tests.

Materials and methods

Entomopathogenic nematodes isolates. The EPNs isolates used in this work were obtained by the Universidade Federal de Lavras (UFLA), MG, Embrapa, Trigo, RS, and the Instituto Biológico de Campinas, SP, Brazil. Isolates were reared in *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) last-instar larvae, according to the methodology described by Molina and López (2001). Ten *G. mellonella* larvae were placed in Petri dish (9 cm of diameter), containing two sheets of filter paper and 2 mL of aqueous suspension at approximately 500 Infective Juveniles (IJs)/mL. Petri dishes were kept for three days in climatic chamber at 25 ± 1 °C, with relative humidity (RH) of 70 ± 10 %, and 12 h photoperiod, when dead larvae and those with symptoms of infection by nematodes were transferred to Petri dishes with filter paper and kept in climatic chamber for five more days.

After this period, larvae were transferred to White trap (White 1927), and the suspension was taken daily and stored in a Becker containing distilled water and pump for aeration for at the most one week before being used in the tests.

Selection of entomopathogenic nematodes isolates. The nine isolates used for the selection test (Table 1) had been previously reared by the method described by Molina and López (2001). The experiment was carried out in 100 mL plastic cups containing 7 g of sterile vermiculite, which had previously received two seeds of corn, and were maintained at room temperature for about a week, until seeds germination.

When plants reached 10 cm, nematodes isolates were quantified and applied over the vermiculite at the concentration of 100IJs/cm² (the control treatment received only distilled water), standardizing the percentage of humidity of vermiculite to 20 % (1.4 mL/plastic cup). Afterwards, one of 3rd instar larvae of *A. ipsilon* was put in each cup, totaling 20 larvae per treatment.

Treatments were kept in climatic chamber at 25 ± 1 °C, RH of 70 ± 10, and 12 hours photoperiod, for five days. After that, the number of dead insects was observed. Dead larvae were dissected under a stereoscopic microscope to confirm mortality by nematodes. The percentage of mortality was calculated in each treatment, considering 20 larvae as 100 %.

Tests with immature stages of *A. ipsilon*. Susceptibility at different stages (2nd, 3rd, 4th, 5th and 6th instars and pupae) of *A. ipsilon* was evaluated for the three nematode isolates that presented greater pathogenicity in the selection tests of isolates IBCB-n02 (*Steinernema carpocapsae*), IBCB-n05 (*Heterorhabditis indica*) and GL (*Heterorhabditis amazonensis*).

The test was carried out in 100 mL plastic cups containing 7 g of sterile vermiculite, by placing one larva of *A. ipsilon* on the surface together with a piece of diet (modified of Greene *et al.* 1976) for feeding. Each treatment was replicated four times, and each plot corresponded to ten cups containing one larvae/cup. The pupae were buried into the vermiculite without diet. Afterward, nematode suspension was applied with pipette on the vermiculite surface, at the concentration of 100IJs/cm² (only distilled water was used in the control treatment), standardizing humidity to 20 % (1.4 mL/plastic cups).

Cups were kept in climatic chamber at 25 ± 1 °C, RH 70 ± 10, with 12 hours photoperiod, and the test was carried out

Table 1. List of the isolates of entomopathogenic nematodes (genera/species), place of origin, institution and mortality rate caused in *Agrotis ipsilon* under laboratory conditions.

Isolates	Genus/ species	LOrigin	Institution	Mortality (%)
GL	*Heterorhabditis amazonensis*	Lavras – MG – Brazil	UFLA	90
RSC05	*Heterorhabditis amazonensis*	Benjamin Constant – AM – Brazil	UFLA	80
JPM4	*Heterorhabditis* sp.	Lavras – MG – Brazil	UFLA	80
CH3	*Steinernema* sp.	Campinas – SP - Brazil	Inst. Biológico	100
IBCB-n02	*Steinernema carpocapsae*	Flórida – USA	Inst. Biológico	90
IBCB-n05	*Heterorhabditis indica*	Itapetininga – SP – Brazil	Inst. Biológico	90
IBCB-n40	*Heterorhabditis* sp.	Taboporã – SP – Brazil	Inst. Biológico	80
IBCB-n44	*Heterorhabditis* sp.	Sta. Adélia – SP – Brazil	Inst. Biológico	80
NEPET11	*Heterorhabditis* sp.	Palmeira das Missões – RS – Brazil	Embrapa Trigo	10

in a completely randomized design, in a factorial scheme 4 x 5, considering three isolates and the control (4), and five instars (5). All data were subjected to homogeneity (Hartley) and normality (Shapiro-Wilk) tests to verify the assumptions of parametric statistics. Data were subjected to analysis of variance (ANOVA), and means were compared by the Tukey test at 5 % probability, using the software SISVAR 5.4 (Ferreira 2011).

Concentrations test. The three isolates that proved to be more virulent to *A. ipsilon,* in the selection test of isolates, were selected (IBCB-n02, IBCB-n05 and GL), using the same methodology of the test with immature stages. However, only 3rd larvae instar was considered, with concentrations of 0 (control treatment), 50, 100, 150, and 200 IJs/cm^2.

The test was carried out in completely randomized design, and all data were subjected to homogeneity (Hartley) and normality (Shapiro-Wilk) tests to verify the assumptions of parametric statistics. Data were subjected to analysis of variance (ANOVA) and regression analysis, using the statistical software SISVAR 5.4 (Ferreira 2011) to determine the regression curve and the equation of regression for the interval evaluated in the study.

In vivo **production of isolates *Steinernema carpocapsae* (IBCB-n02), *Heterorhabditis indica* (IBCB-n05) and *Heterorhabditis amazonensis* (GL) in *A. ipsilon* larvae.** Plastic Petri dishes (9 cm of diameter) received two filter papers and 10 *A. ipsilon* larvae, which had been previously weighed. Subsequently, isolates were applied at the concentration of 50 IJs/cm^2 in 2 mL of suspension. Petri dishes were tapped and sealed with PVC film and kept in climatic chamber for 72 hours, at 24 ± 1 °C, RH of 70 ± 10, without photoperiod. After confirmation of mortality, larvae were transferred to new Petri dishes containing only one dry filter paper, and kept for five days under the same conditions. Subsequently, larvae were placed in White traps (one plot per trap). IJs were daily collected in suspension of distilled water and quantified for evaluation of the production. Collection and quantification was repeated until larvae production depletion.

Data were subjected to homogeneity (Hartley) and normality (Shapiro-Wilk) tests to verify the assumptions of parametric statistics. Data were also subjected to analysis of variance and to the Tukey test at 5 % probability, using the software SISVAR 5.4 (Ferreira 2011).

Greenhouse test. For the susceptibility test of *A. ipsilon* under greenhouse conditions, 8 L cup were prepared with soil, at the ratio of 4:1:0.5 soil, substrate (Plantmax®) and vermiculite, totaling 2 L of the mixture of substrates/cup. Five seeds of corn were sown, using the fungicide treatment Maxim XL (fludioxonil 2.5 % + metalaxyl-M 0.1 %) in each plot, and irrigated with 200 mL water a day.

At 10 days after corn emergence, five 3rd instar *A. ipsilon* larvae were placed in each plot, which were separated into groups of 15 plots/treatment for the application of nematodes isolates (IBCB-n02, IBCB-n05 and GL) in aqueous suspension (100 IJs/cm^2) around the plants, using a pipette. Control treatment received only distilled water.

The test was carried out in randomized experimental design. Evaluation occurred at five days after nematodes inoculation, by collecting the dead larvae. Larvae were disinfested with 70 % alcohol and kept in a dry chamber for another three days, when dissection was performed to confirm the death of the insect by the nematode. All data were subjected to homogeneity (Hartley) and normality (Shapiro-Wilk) tests to verify the assumptions of parametric statistics. Data were subjected to analysis of variance (ANOVA) and the Tukey test at 5 % probability, using the software SISVAR 5.4 (Ferreira 2011).

Results and discussion

Selection of entomopathogenic nematodes isolates. Isolate CH3 (*Steinernema* sp.) presented the highest mortality rate in the selection test (100 %) (Table 1). However, rearing this isolate in the laboratory is difficult. In other words, the isolate infects and kills *G. mellonella* larvae, but rearing may not occur, which hinders the production of this isolate to be used in nematode tests. Therefore, isolate CH3 was discarded in subsequent tests.

Other isolates caused between 90 % (IBCB-n02, IBCB-n05, GL) and 80 % of mortality (IBCB-n40, JPM 4, RSC 05 and IBCB-n44), and only the isolate NEPET11 did not cause significant mortality (10 % of mortality) (Table 1).

In relation to susceptibility, Ebssa and Koppenhöfer (2012) tested the isolates *Heterorhabditis bacteriophora*, *H. megidis*, *Steinernema carpocapsae*, *S. feltiae*, *S. kraussei,* and *S. riobrave* in larvae of *A. ipsilon*, and obtained more than 90 % of mortality, except for *S. kraussei,* which caused only 60 % of mortality. In this work, the present selection test of isolates also reported that most isolates caused more than 80 % of mortality.

This variation of susceptibility for different isolates was expected, since different isolates coevoluted with different species of host, which make them more or less virulent to a determined host (Almenara *et al.* 2012).

Table 2. Mortality rate of different instars of *Agrotis ipsilon* after the application of isolates IBCB-n05, GL and IBCB-n02 at the concentration of 100 IJs/cm^2 under laboratory conditions. Temperature of 25 ± 2 °C, RH 70 ± 10 %, 12 h photoperiod.

Treatments	Mortality rate at different instars				
	2º	3º	4º	5º	6º
IBCB-n05	43 ± 8.5 BCa	77 ± 8.5 Bb	83 ± 6.3 Bb	58 ± 7.5 Bab	60 ± 15.8 Bab
GL	48 ± 2.5 Ca	75 ± 2.9 Ba	80 ± 5.8 Ba	60 ± 10.8Ba	75 ± 2.9 Ba
IBCB-n02	15 ± 2.9 ABa	97 ± 2.5 Bd	83 ± 8.5 Bcd	50 ± 4.1 Bbc	45 ± 8.7 Bab
Control	0.0 ± 0.0 Aa	0.0 ± 0.0 Aa	0.0 ± 0.0 Aa	0.0 ± 0.0 Aa	0.0 ± 0.0 Aa

* Means followed by uppercase letters in the columns and lowercase letter in the rows did not significantly differ by the Tukey test (P ≤ 0.05). C.V. 35.59.

Tests with immature stages of *A. ipsilon*. The isolates evaluated (IBCB-n05, IBCB-n02 and GL) caused mortality in immature stages of *A. ipsilon* (2nd, 3rd, 4th, 5th, 6th instar) with similar values to that observed in the selection test, which was performed just with insects in the 3rd stage (Table 2).

The low mortality in the 2nd instar larvae of *A. ipsilon* may be due to the low metabolic activity of the larvae of young instars and lower movement, since EPNs located their hosts by the concentration of CO_2 around them (Nishimatsu and Jacksn 1998). Thus, the low metabolism may have influenced, since it hinders the contact of the nematode with the larvae, which explains the low mortality.

The 3rd and 4th instars presented the highest mortality rate, reaching 97 % in larvae of the 3rd instar and 83 % in larvae of the 4rd for isolate IBCB-n02 (*S. carpocapsae*). Ebssa and Koppenhöfer (2012) reported similar results in tests with different instars of *A. ipsilon* for the species *S. carpocapsae,* with higher mortality rate for the larvae at the 4th and 5th instars. Conversely, the same authors reported that the 6th instar was less susceptible, differing from the results observed in the present study, in which the lowest mortality rate was obtained in the 2rd instar. Larvae of some Lepidoptera increase susceptibility when they are older; this is because they are bigger, attracting more nematodes, and present more body openings (mouth, anus, and spiracle), making them more susceptible to infective juveniles (Smiths *et al*. 1994, Shannag *et al*. 1994). However, in other lepidopteran species, older instars and the pupae are less susceptible (Glazer and Navon 1990; Shannag *et al*. 1994; Baur *et al*. 1998). In relation to *A. ipsilon*, according to Ebssa and Koppenhöfer (2012), the reduced body size of the 2nd instar larvae is the main factor for the low mortality rate, since the small size of the larvae makes it difficult for the IJs to access the larvae's body.

Pupae mortality rate observed in the test was not significant for any of the isolates evaluated (5 % for IBCB-n02 and 0 % for IBCB-n05 and GL).

Likewise, in the study developed by Chambers *et al*. (2010) with *Cydia pomonella* (Lepidoptera: Tortricidae) (apple moth), the pupae were also less susceptible than the larvae, and in the work of Ebssa and Koppenhöfer (2012), mortality rate of *A. ipsilon* pupae did not surpass 50 %, even for the isolates that caused high mortality rate in the larvae.

Among the alternatives proposed for the low susceptibility of *A. ipsilon* pupae, it can be suggested that the lower value of CO_2 at this stage may decrease the attraction, or even the displacement the IJ toward the insect (Lewis *et al*. 1993). In addition, according to Ebssa and Koppenhöfer (2012), *A. ipsilon* pupae stage occurs in the soil, causing coevolution of this stage together with the EPNs, which could have led to the development of behavioral, physical or physiological defense mechanisms to these entomopathogenic agents.

Concentrations test. All the isolates were pathogenic to *A. ipsilon* at all the concentrations, with mortality rate between 10 and 100 % (Fig. 1). However, mortality data from the isolate GL did not meet the assumptions of normality and homoscedasticity, and therefore were not subjected to regression analysis. Isolate IBCB-n02 (*S. carpocapsae*)

showed increasing mortality rate of the larvae stage, according to the increase of the concentration used in the test, and CL_{99} was estimated at a concentration of 140 IJs/cm^{2}. The isolate IBCB-n05 (*H. indica*) had CL_{99} estimated at a concentration of 190 IJs/cm^{2}. For both isolates (IBCB-n02 and IBCB-n05), concentrations can be considered as high. This is confirmed with a study developed by Alves *et al*. (2017), CL_{99} of the isolate CB40 (*Heterorhabditis* sp.) for *G. mellonella* was of 9 IJs/cm^{2}. However, *G. mellonella* is considered as an insect highly susceptible to most entomopathogenic nematode isolates.

In a study developed by Andaló *et al*. (2010) with *Spodoptera frugiperda* (Smith, 1797) (Lepidoptera: Noctuidae), the number of IJs to cause maximum mortality was of 223 IJs/larvae for the isolate RSC02 (*Heterorhabditis* sp.) and of 218 IJs/larvae for *S. arenarium*, which are higher values than those observed in this work.

Moreover, according to another study on nematodes for biological control of Lepidoptera, specially *S. frugiperda*, mortality rate of larvae increased with the increase in the concentration (Polanczyk and Alves 2005), corroborating the present results.
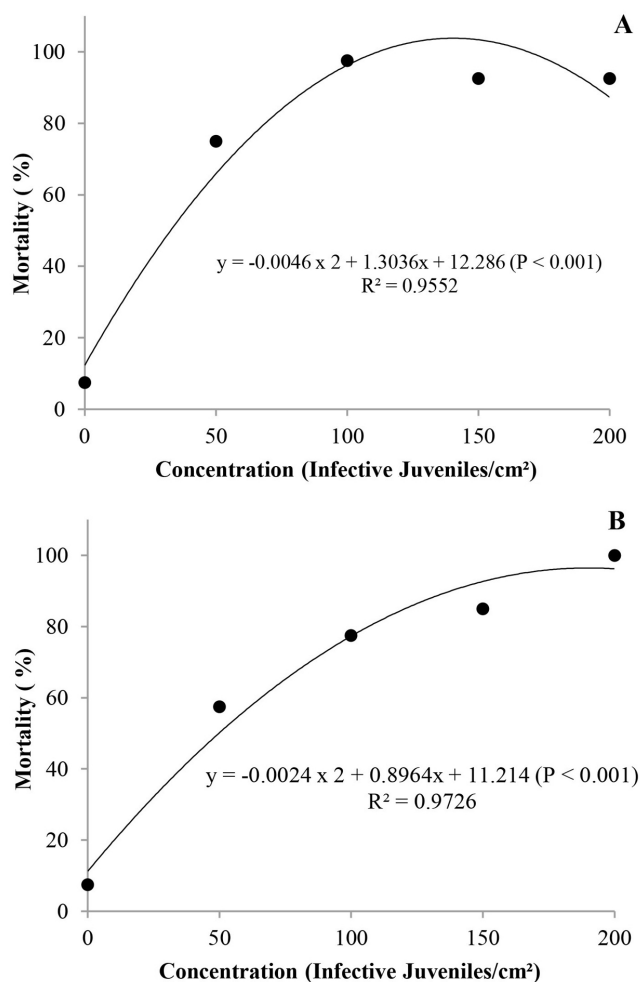

A.

$$y = -0.0046 x^2 + 1.3036x + 12.286 \ (P < 0.001)$$
$$R^2 = 0.9552$$

B.

$$y = -0.0024 x^2 + 0.8964x + 11.214 \ (P < 0.001)$$
$$R^2 = 0.9726$$

Figure 1. Mortality of *Agrotis ipsilon* larvae caused by different isolates of entomopathogenic nematodes at different concentrations (IJs/cm^{2}), under laboratory conditions (T = 25 ± 1 °C, RH 90 ± 10 %, and 12 h photoeriod). **A.** IBCB-n02 *Steinernema carpocapsae*. **B.** IBCB-n05 *Heterorhabditis indica*.

***In vivo* production of isolates *Steinernema carpocapsae* (IBCB-n02), *Heterorhabditis indica* (IBCB-n05) and *Heterorhabditis amazonensis* (GL) in *A. ipsilon* larvae.** At the end of the production test, the isolates IBCB-n02 (*S. carpocapsae*), IBCB-n05 (*H. indica*), and GL (*H. amazonensis*) produced 4.7×10^{-4} IJs/larvae, 2.3×10^{-4} IJs/larvae, and 3.3×10^{-4} IJs/larvae, respectively. Therefore, no difference was reported between treatments (Table 3).

No production tests of EPNs in *A. ipsilon* have been reported yet. Nevertheless, when compared with production data obtained with *G. mellonella*, the values reported in this work are considered low. Guide *et al.* (2016) observed that the isolates NEPET11 (*Heterorhabditis* sp.) and RSC05 (*Heterorhabditis amazonensis*) presented values of 7×10^{-4} IJs/g larvae and 7.2×10^{-4} IJs/g larvae, respectively. In addition, Bortoluzzi *et al.* (2013) evaluated two isolates in *G. mellonella* and observed 2.2×10^{-5} IJs/g larvae for CB24 (*Heterorhabditis* sp.), and 2.2×10^{-5} IJs/g larvae for CB40 (*Heterorhabditis* sp.)

Galleria mellonella larvae are susceptible to entomopathogenic nematodes and are frequently used for *in vivo* production of these nematodes (Gaugler and Han 2002). Conversely, larvae that spend part of their life cycle in contact with the soil, as in the case of *A. ipsilon*, have defense mechanisms against these agents, which can affect the development and reproduction of EPNs in their interior. Thus, Molina *et al.* (2004) evaluated the production of three nematodes isolates in four different host (*G. mellonella*, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae)*, S. frugiperda* and *Bombyx mori* L. (Lepidoptera: Bombycidae), and observed great difference in production. In addition, *Steinernema glaseri* did not rear in *T. molitor, S. frugiperda* and *B. mori*, but it satisfactorily reared in *G. mellonella*. All isolates were able to rear in *A. ipsilon*, suggesting that the insect has mechanisms of resistance that can influence the rearing of the isolates, but cannot prevent EPNs from reproducing inside the larvae.

According to Stuart (2006), there are differences in relation to the virulence of EPNs, which can be attributed to the specificity, morphology, origin, and even the genetic variability of these isolates.

Greenhouse test. The isolate IBCB-n02 reduced the survival rate of the 3rd instar larvae of *A. ipsilon* in the soil. Only this isolate differed from the control treatment under greenhouse conditions (Fig. 2).

During the experiment, larvae caused damage to the corn plants by cutting the stems, at not only in control, but also in the nematodes treatments.

These results can be compared with those obtained by Riga *et al.* (2001), who tested two isolates of
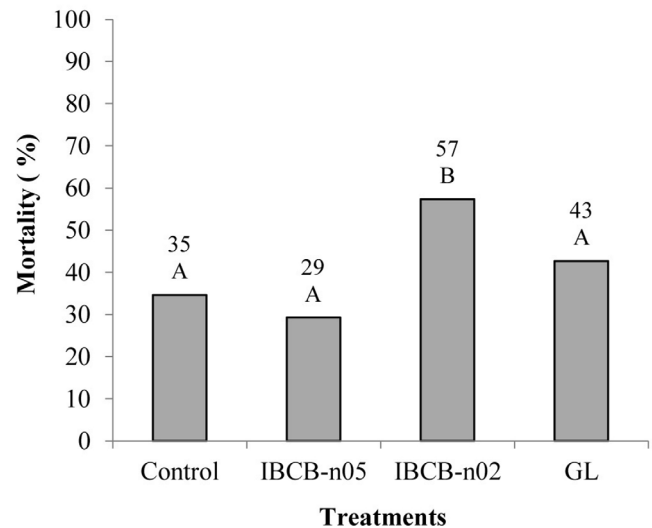

Figure 2. Mortality rate of *Agrotis ipsilon* larvae caused by different isolates of entomopathogenic nematodes under greenhouse conditions. Columns followed by the same uppercase letter did not significantly differ by the Tukey test ($P \le 0.05$). C.V. 63.24.

Steinernema against four corn pests, being one of the the noctuidae *S. frugiperda,* under greenhouse conditions. The authors reported that these isolates decreased the damage against corn plants, when compared with the control treatment (without nematode).

In a study developed by Ebssa and Koppenhöfer (2012), the five isolates (*H. megidis, H. bacteriophora, S. carpocapsae, S. riobrave* and *S. feltiae*) caused mortality in *A. ipsilon* under greenhouse conditions, both in larvae at the 3rd and 5th instars. According to the authors, the highest mortality rates were obtained at concentrations of 200IJs/cm^2, which corresponds to twice the concentration used in this work. According to these authors, the time of evaluation was determinant in the mortality rate, and more significant values were observed when the evaluation was carried out at seven days after inoculation.

Factors such as temperature influenced the infectivity of the entomopathogenic nematodes. Moreover, species react differently, *i.e.*, Heterorhabditidae are more sensitive to temperature than Steinernematidae, which might have caused lower mortality rate in the treatment with *Heterorhabditis* (Flanders *et al.* 1996).

In the present study, only the isolate IBCB-n02 (v) was able to cause significant mortality in the greenhouse test, which reinforces this statement. Results suggest that this isolate stood out in relation to GL and IBCB-n05, which are of the family Heterorhabditidae.

Conclusion

The isolate IBCB-n02 (*Steinernema carpocapsae*) is the most indicated to be used in future studies that aim at *A. ipsilon* control.

Table 3. *In vivo* production of entomopathogenic nematodes (IJs/larvae) in *Agrotis ipsilon*. Temperature of 25 ± 2 °C, RH 70 ± 10 %, and 12 h photoperiod.

Isolates	IJs/larvae	Mean weight of larvae
IBCB-n02	$4.7 \times 10^{-4} \pm$ A*	0.26 g
IBCB-n05	2.3×10^{-4} A	0.24 g
GL	3.3×10^{-4} A	0.28 g

* Means followed by the same uppercase letter in the columns did not significantly differ by the Tukey test ($P \le 0.05$). C.V. 30.95.

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