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Selection of entomopathogenic nematodes and evaluation of their compatibility with cyantraniliprole for the control of *Hypothenemus hampei*

Seleção de nematoides entomopatogênicos e avaliação da sua compatibilidade com ciantraniliprole visando o controle de *Hypothenemus hampei*

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Abstract

Attack by the coffee berry borer *Hypothenemus hampei* causes significant damage to coffee crops because it affects the quality of the coffee fruit during different developmental stages, which results in production losses. Control of the borer is difficult owing to its cryptic behavior and the fact that it spends its entire life cycle inside the coffee berries. This makes it difficult for natural enemies to reach it, as well as for it to come into contact with chemical insecticides. The objective of the present study was to select and evaluate the virulence of entomopathogenic nematodes (EPNs) on the coffee berry borer *H. hampei* and their compatibility with the insecticide cyantraniliprole under laboratory conditions. Initially, the pathogenicity and virulence of 16 isolates of *Steinernema* and *Heterorhabditis* towards coffee berry borer larvae and adults were evaluated. The most virulent isolates to both larvae and adults were determined by topical inoculation tests in coffee fruits (berries) infested by the insect, using a concentration of 100 infective juveniles (IJs)/fruit. The same isolates were also evaluated for viability and infectivity when combined with cyantraniliprole. The isolates *S. feltiae* (IBCB-n 47) and *Heterorhabditis amazonensis* (GL) displayed the highest virulence towards adults (54%). For larvae, we observed a high virulence of *S. feltiae*, *Heterorhabditis amazonensis*, *Heterorhabditis indica*, *Heterorhabditis* sp. (JPM4), *Heterorhabditis* sp. (NEPET 11), *Heterorhabditis* sp. (IBCB-n 46), and *Heterorhabditis* sp. (IBCB-n 44) that promoted 100% mortality. Regarding the topical inoculation test on infested fruits, *S. feltiae* and *Heterorhabditis* sp. (IBCB-n 46) were unable to penetrate the fruit through the hole made by the borer, infect, and cause the death of insects. Cyantraniliprole formulation affected the viability of IJs of *S. feltiae* and *Heterorhabditis* sp. (IBCB-n 46), mainly after 48 h of exposure.

Key words: Biological control. *Heterorhabditis*. *Steinernema*. *Coffea arabica*. *Coffea canephora*.

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Resumo

A broca-do-café *Hypothenemus hampei* causa significativos prejuízos a esta cultura, pois seu ataque afeta a qualidade dos frutos de café em seus diferentes estádios de desenvolvimento e consequentemente leva a perdas na produção. O controle é dificultado devido ao seu comportamento críptico, uma vez que passa todo o ciclo dentro dos frutos de café, dificultando a ação de inimigos naturais, bem como o contato com inseticidas químicos. O objetivo deste estudo foi avaliar a virulência de nematoides entomopatogênicos (NEPs) sobre a broca-do-café *H. hampei* e a sua compatibilidade com o inseticida ciantraniliprole em condições de laboratório. Inicialmente, foram avaliadas a patogenicidade e virulência de 16 isolados de *Steinernema* e *Heterorhabditis* para larvas e adultos de *H. hampei*. Os isolados mais virulentos para larvas e adultos foram utilizados no teste de inoculação tópica em frutos de café infestados pelo inseto, utilizando uma concentração de 100 juvenis infectantes (JIs)/fruto. Os mesmos isolados também foram avaliados quanto à viabilidade e à infectividade quando combinados com ciantraniliprole. Os isolados *Steinernema feltiae* (IBCB-n 47) e *Heterorhabditis amazonensis* (GL) foram os que apresentaram maior porcentagem de mortalidade sobre os adultos (54%). Para larvas, observou-se alta virulência dos isolados *S. feltiae*, *H. amazonensis*, *H. indica*, *Heterorhabditis* sp. (JPM4), *Heterorhabditis* sp. (NEPET11), *Heterorhabditis* sp. (IBCB-n 46) e *Heterorhabditis* sp. (IBCB-n 44) que promoveram 100% de mortalidade. Quanto ao teste de inoculação tópica sobre frutos infestados, observou-se que tanto *S. feltiae* quanto *Heterorhabditis* sp. (IBCB-n 46) não foram capazes de penetrar o fruto pelo orifício feito pela broca, infectar e causar a morte dos insetos. A formulação de ciantraniliprole afetou a viabilidade dos JIs de *S. feltiae* e *Heterorhabditis* sp. (IBCB-n 46), principalmente após 48 horas de exposição.

Palavras-chave: Controle biológico. *Heterorhabditis*. *Steinernema*. *Coffea arabica*. *Coffea canephora*.

Introduction

The coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae) is considered one of the most important coffee pests. It is found in almost all coffee producing regions and directly attacks the fruit of the coffee on which it feeds, develops, and reproduces. The damage can be seen in all stages of maturation and is characterized by weight loss and depreciation of the grain (VEGA et al., 2009). In addition, attacks may facilitate the entry of microorganisms that develop in grains, affecting the quality of the final coffee product (VEGA et al., 2009, 2014). The annual losses caused by this insect in Brazil have already exceeded US$300 million (OLIVEIRA et al., 2013).

The methods used to control this insect are based on the use of synthetic insecticides (JARAMILLO et al., 2011); however, alternative controls are required. Previous studies on the use of biological controls have been performed, such as the use of plant extracts of *Tephrosia purpurea* (leaf) and *Moringa oleífera* (seed) that showed lethal activity against *H.hampei* (ZORZETTI et al., 2012). The use of parasitoids of the family Bethylidae has also been evaluated; however, results have not been promising, because of the difficulty in breeding, mass production, adaptation, and non-significant levels of parasitism (VEGA et al., 2009). Entomopathogenic fungi such as *Beauveria bassiana* are also important control agents of adults (MOTA et al., 2017); however, they need ideal conditions to remain viable in the environment, which hampers their use (NEVES; HIROSE, 2005).

Insect control with entomopathogenic nematodes (EPNs) can be a viable, efficient, and safe alternative. These entomopathogens possess several characteristics, such as the ability to search the host and compatibility with plant protection products that make them advantageous compared to other biological control agents (LEWIS et al., 2006). In addition, studies have demonstrated that EPNs are able to penetrate the coffee fruit that have fallen on the ground through the orifice made by the drill, thus presenting good results in the control of *H.*

hampei (MOLINA; LÓPEZ, 2009; BENAVIDES-MACHADO et al., 2010; MANTON et al., 2012).

Within the groups of EPNs considered important in pest control, the genus *Heterorhabditis* Poinar, 1976 and *Steinernema* Travassos, 1927 stand out because they are known for aspects related to ecology, pest action strategies, large-scale production, and application conditions.

Given that infested coffee fruits that have fallen on the ground and those left on the plant after harvesting are the main sources of reinfestation during the next harvest (BENAVIDES-MACHADO et al., 2010) and the difficulty in controlling this pest due to its cryptic habit, the use of EPNs may be a promising alternative to traditional methods.

Thus, the objective of the present study was to assess the potential use of EPNs as an alternative for biological control of the coffee berry borer, *H. hampei*, and the compatibility of these agents with the insecticide cyantraniliprole.

Materials and Methods

Experiments were carried out under laboratory conditions. The coffee borer was obtained from the Laboratory of Entomology of the Agronomic Institute of Paraná and was maintained in a climatic chamber (25 ± 2 °C, relative humidity (RH): 60 ± 10%, no light) and developed on an artificial diet adapted from Villacorta and Barrera (1993), with the following modifications: 150 g of coffee powder (raw grain) and 0.5 g of Vanderzant vitamin mixture for insects were added.

Obtaining and maintaining isolates of entomopathogenic nematodes

Sixteen isolates of EPNs were obtained from the database of entomopathogens from the Laboratory of Entomology and Microbial Control of the State University of North Paraná (Cornélio Procópio, PR) and the Biological Institute (Campinas, SP) (Table 1).

Table 1. Isolates of entomopathogenic nematodes of the genera *Steinernema* and *Heterorhabditis* used in the selection test against larvae and adults of *Hypothenemus hampei* under laboratory conditions.

Species	Isolate	Origin
Heterorhabditis amazonensis	RSC 05	Benjamin Constant, AM, Brazil
Heterorhabditis indica	IBCB-n 05	Itapetininga, SP, Brazil
Heterorhabditis bacteriophora	HP 88	New Jersey, USA
Heterorhabditis sp.	NEPET 11	Palmeira das Missões, RS, Brazil
Heterorhabditis amazonensis	GL	Lavras, MG, Brazil
Heterorhabditis sp.	JPM 4	Lavras, MG, Brazil
Heterorhabditis sp.	IBCB-n 40	Taboporã, SP, Brazil
Heterorhabditis sp.	IBCB-n 46	Santo Antônio de Posse, SP, Brazil
Heterorhabditis sp.	IBCB-n 44	Santa Adélia, SP, Brazil
Steinernema sp.	IBCB-n 27	Mogi Guaçu, SP, Brazil
Steinernema carpocapsae	IBCB-n 02	Flórida, USA
Steinernema diaprepesi	AM 163	Sinop, MT, Brazil
Steinernema rarum	PAM	Aceguá, RS, Brazil
Steinernema feltiae	IBCB-n 47	Flórida, USA
Steinernema puertoricense	CER-n 125	Rio Verde, GO, Brazil
Steinernema glaseri	IBCB-n 01	Botucatu, SP, Brazil

Breeding was performed with the *in vivo* method adapted from the methodology described by Molina and López (2001), using the last instar larvae of *Galleria mellonella* (Linnaeus) (Lepidoptera: Pyralidae) from established breeders. After the emergence of the infective juveniles (IJs), they were collected and placed in distilled water, stored in plastic pots, and maintained in a climatic chamber at 18 ± 1 °C without photoperiod for up to 3 days.

Virulence of entomopathogenic nematodes against H. hampei adults

Each isolate (treatment) was replicated five times. The experimental unit corresponded to a 5.5 cm diameter glass Petri dish containing one unsexed insect and two dry filter paper sheets on the base and plate lid. The isolates were then applied at a concentration of 100 IJs/cm^2 on the base and lid papers of the dish with the aid of a micropipette.

After application, the dishes were capped, sealed with plastic film, and maintained in a climatic chamber at 25 ± 1 °C, RH: 70 ± 10%, and in scotophase. An additional treatment (control) was included, which contained only 1 mL of distilled water. Evaluation occurred 5 days after inoculation and quantified the number of insects killed by the nematodes. To confirm mortality, the insects were dissected and displacement of the head from the thorax was recorded.

The experiment was designed in a randomized experimental design. The data were subjected to analysis of variance, transformed into root^2 (x + 0.5) to meet the assumptions of a parametric analysis, and the means were compared by the Scott-Knott test with 5% significance using the SISVAR 5.4 software program (FERREIRA, 2011).

Virulence of entomopathogenic nematodes against H. hampei larvae

The treatments consisted of four replications. Each replication corresponded to a Petri dish of 5.5 cm in diameter, containing ten second instar larvae, two filter papers in the base, and the diet (the same as that used for breeding). Then, for each isolate, 1 mL of suspension containing the isolate at a concentration of 100 IJs/cm^2 was applied on the filter papers with the aid of a micropipette.

After inoculation, the plates were capped and maintained in a climatic chamber at 25 ± 1°C, RH: 70 ± 10%, and without photophase. An additional treatment (control) was included that only received 1 mL of distilled water. Evaluation occurred 5 days after inoculation and quantified the number of dead larvae.

The experiment was designed in a randomized experimental design. The statistical procedure performed with the data from the screening test with larvae of the coffee berry borer was the same as that for the adults. However, the processing of data was not necessary.

Application of infective juveniles on infested coffee fruits

In the present study, the isolates *S. feltiae* (IBCB-n 47) and *Heterorhabditis* sp. (IBCB-n 46), which were among the most virulent of the selection tested, were used.

The following treatments were performed: nematode isolates applied in aqueous suspension; nematode isolates applied with 0.5% xanthan gum fixative to avoid dehydration; and the control treatment, in which only distilled water was applied. Each treatment consisted of eight replicates and the experimental unit was a 9 cm diameter glass Petri dish containing 10 g of previously autoclaved and 80 °C oven-dried vermiculite as the base for the fruit and two infested fruits. A total of 100 IJs were applied on each fruit with the help of a micropipette. The plates were capped and maintained in a climatic chamber at 25 ± 1 °C and without photophase for 5 days.

To evaluate the mortality of insects, the fruits were washed superficially with distilled water to eliminate the EPNs that had not yet penetrated. Then, dissection of the fruits occurred. The numbers of IJs inside the fruits, apart from eggs, and all stages of the insect (e.g., larva, pupa, and adult) were counted, and the dead drills dissected with the aid of a stereoscope microscope to confirm parasitism by EPNs.

Compatibility of EPNs isolates with cyantraniliprole

The assessment of the compatibility of *S. feltiae* (IBCB-n 47) and *Heterorhabditis* sp. (IBCB-n 46) with the insecticide cyantraniliprole was performed based on the methodology described by Vainio (1992) and modified by Negrisoli Júnior et al. (2008).

One liter of product formulation was prepared with twice the recommended dose of cyantraniliprole (1,750 mL of commercial product/400 L of water). Thus, 8.75 mL of commercial product were added to 1,000 mL of water. A 1 mL sample of this solution was distributed in five glass tubes per treatment. A sample of 1 mL of distilled water containing 2,500 IJs was added to each tube, thus re-establishing the dose recommended for field application. As a control, 1 mL of suspension of isolates was mixed with 1 mL of distilled water. The tubes were stored in a climatic chamber at 22 ± 1 °C, RH of 70 ± 10%, and without photophase.

The viability of nematodes was evaluated at 24 h and 48 h after exposure to the product. Thus, 50 µL of the suspension in each tube (replicate) was removed and 100 IJs were observed under a stereoscope microscope to determine viability. All immobile nematodes and those that did not respond to touch with a stylus were considered dead and those who displayed movement were considered alive.

The infectivity of IJs was evaluated during the same period as viability. The tubes were supplemented with 3 mL of distilled water and left to settle for 30 min in a fridge at 10 °C, after which the supernatant (approximately 3 mL) was discarded. This rinsing procedure was repeated three times to remove the maximum amount of insecticide. After the last wash, 1 mL (approximately 1,000 IJs) was removed from the bottom of each tube and applied on five Petri plates containing two filter papers and ten last instar larvae of *G. mellonella*.

The plates were maintained in a climatic chamber at 22 ± 1°C for 5 days. After this period, the dead larvae were transferred to plates containing a dry filter paper and maintained in the dark for over 72 h, and were then dissected with the aid of a stereoscope microscope to confirm death by nematodes.

The viability of nematodes after exposure to cyantraniliprole and mortality of larvae infected by EPNs were submitted to analysis of variance and the means were compared by the Tukey's test ($P \leq 0.05$) using the SISVAR 5.4 software program (FERREIRA, 2011).

Results and Discussion

Selection test with adults

All isolates were pathogenic; however, they displayed differences in virulence. Mortality ranged from 2% to 54% (Table 2).

The difference in virulence among isolates, even of the same species, can be justified by several factors. Different isolates can be adapted to different climatic conditions or develop host specificity (ALVES et al., 2009). According to a study by Almenara et al. (2012), such variation in susceptibility is expected because different isolates coevolved with different host species and, therefore, have characteristics that make them virulent to a greater or lesser degree on certain insects.

Table 2. Mortality (%) of adults of *Hypothenemus hampei* caused by entomopathogenic nematodes (*Steinernema* spp. and *Heterorhabditis* spp.) under laboratory conditions (25 ± 1 ºC, RH 70 ± 10%, and without photophase).

Species	Isolate	Mortality (%) ± (SEM)
Steinernema feltiae	IBCB-n 47	54.0 ± 8.1 a*
Heterorhabditis amazonensis	GL	54.0 ± 7.5 a
Heterorhabditis sp.	IBCB-n 46	50.0 ± 7.1 a
Heterorhabditis bacteriophora	HP 88	46.0 ± 9.8 a
Heterorhabditis indica	IBCB-n 05	44.0 ± 13.6 a
Steinernema diaprepesi	AM 163	44.0 ± 9.8 a
Steinernema glaseri	IBCB-n 01	42.0 ± 7.3 a
Heterorhabditis sp.	IBCB-n 40	40.0 ± 7.1 a
Heterorhabditis sp.	IBCB-n 44	40.0 ± 8.4 a
Steinernema rarum	PAM	38.0 ± 6.6 a
Heterorhabditis amazonensis	RSC 05	24.0 ± 7.5 b
Heterorhabditis sp.	JPM 4	22.0 ± 5.8 b
Steinernema carpocapsae	IBCB-n 02	22.0 ± 7.3 b
Heterorhabditis sp.	NEPET 11	16.0 ± 6.0 c
Steinernema sp.	IBCB-n 27	6.0 ± 4.0 d
Steinernema puertoricense	CER-n 125	4.0 ± 2.4 d
Control	-	2.0 ± 2.0 d
		CV (%) = 30.97

*Means followed by the same letter do not differ by the Skott-Knott test ($p \leq 5\%$) with data transformed into root^2 (x + 0.5).

From a previous study (CASTILLO; MARBÁN-MENDOZA, 1996) that assessed the pathogenicity of eight isolates of EPNs on coffee berry borer adults in Petri plates containing diet, the isolates *Heterorhabditis* LIM-1, DOM-8, and PC-3, and the commercial isolate *S. carpocapsae* (Biosys) were found to be the most virulent to insects, with a mortality percentage above 50%. In the present study, a similar mortality percentage was observed for some of the *Heterorhabditis* isolates; however, *S. carpocapsae* caused lower mortality (22%) than that observed by the authors.

Benavides-Machado et al. (2010) evaluated the virulence of isolates of nematodes on coffee berry borer adults and observed that all treatments were pathogenic to the insects, whereas the mortality 5 days after the application ranged from 3.6% to 24.6%, with *H. bacteriophora* being the most virulent isolate (24.6%). These results are lower than those found in the present study because *H. bacteriophora* caused 46% mortality in insects (Table 2).

It was also observed that, in general, the isolates of the genus *Heterorhabditis* were more virulent to adults of *H. hampei*. Identical results were observed by Castillo and Marbán-Mendoza (1996) and Benavides-Machado et al. (2010) in studies conducted with coffee berry borer adults.

Sepúlveda-Cano et al. (2008) performed tests with *Cosmopolites sordidus* (Coleoptera: Curculionidae) adults and observed higher mortality with *H. bacteriophora* (58%) than with *S. carpocapsae* (40%), which shows some similarity with the present study where *S. carpocapsae* was less virulent to adults of the coffee berry borer compared to *H. bacteriophora* (22.0% and 46.0%, respectively).

In previous studies by Tavares et al. (2007) and Giometti et al. (2011), *Heterorhabditis* isolates displayed higher virulence that *Steinernema* on sugarcane weevil adults, *Sphenophorus levis* (Coleoptera: Curculionidae).

Other studies carried out under similar conditions evidenced the susceptibility of curculionids to the nematodes of the genus *Steinernema*, but preferentially to isolates of the genus *Heterorhabditis* (SHAPIRO-ILAN et al., 2000; STUART et al., 2004, SEPÚLVEDA-CANO et al., 2008). The higher susceptibility to *Heterorhabditis* can be explained in part by the fact that they have small appendages in the cephalic region that allow them to penetrate the insect by rupturing their integument (GEDEN et al., 1985).

Adults of *H. hampei* infected by EPNs did not present the typical staining of infection for either nematode genus and some infected adults had displacement of the head from the thorax. This latter feature was also observed by Benavides-Machado et al. (2010) who emphasized the fact that the cause of this displacement is probably due to the pressure generated by the nematode during its development inside the insect.

Selection test with larvae

All isolates were pathogenic to larvae of *H. hampei*, however, the virulence varied. The mortality ranged from 15% to 100%. It was also observed that twelve isolates caused mortality above 80%, and seven of these (six of the genus *Heterorhabditis* and one of the genus *Steinernema*) were able to kill 100% of the larvae (Table 3).

Table 3. Mortality (%) of larvae of *Hypothenemus hampei* caused by entomopathogenic nematodes (*Steinernema* spp. and *Heterorhabditis* spp.) under laboratory conditions (25 ± 1 ºC, RH: 70 ± 10%, and without photophase).

Species	Isolate	Mortality (%) ± (SEM)
Heterorhabditis indica	IBCB-n 05	100.0 ± 0.0 a*
Heterorhabditis sp.	JPM 4	100.0 ± 0.0 a
Heterorhabditis sp.	NEPET 11	100.0 ± 0.0 a
Heterorhabditis sp.	IBCB-n 44	100.0 ± 0.0 a
Heterorhabditis sp.	IBCB-n 46	100.0 ± 0.0 a
Heterorhabditis amazonensis	RSC 05	100.0 ± 0.0 a
Steinernema feltiae	IBCB-n 47	100.0 ± 0.0 a
Heterorhabditis amazonensis	GL	97.0 ± 2.5 a
Steinernema rarum	PAM	90.0 ± 5.8 b
Heterorhabditis sp.	IBCB-n 40	87.5 ± 6.3 b
Steinernema carpocapsae	IBCB-n 02	87.5 ± 2.5 b
Steinernema sp.	IBCB-n 27	85.0 ± 2.9 b
Heterorhabditis bacteriophora	HP88	70.0 ± 10.0 b
Steinernema glaseri	IBCB-n 01	35.0 ± 5.0 c
Steinernema diaprepesi	AM 163	32.0 ± 2.5 c
Steinernema puertoricense	CER-n 125	15.0 ± 5.0 d
Control	-	5.0 ± 2.9 d
		CV (%) = 10.12

*Means followed by the same letter did not differ by the Scott-Knott test ($p \leq 5\%$).

When evaluating the virulence of EPNs on larvae of the coffee berry borer, Benavides-Machado et al. (2010) observed that all isolates were pathogenic and species of the genus *Steinernema* caused mortality between 75% and 90%, whereas those of the genus *Heterorhabditis* caused between 36.5% and 42% mortality. These data differ from the values observed in the present study, as for the genus *Steinernema*, the mortality ranged between 15% and 100% and for *Heterorhabditis* mortality ranged between 70% and 100%.

The *H. hampei* larvae were more susceptible to nematodes than the adults were, reinforcing a trend of higher susceptibility to EPNs that has been verified previously for coffee berry borer larvae (MOLINA; LÓPEZ, 2009; BENAVIDES-MACHADO et al., 2010) and several other coleopterans of the family Curculionidae in laboratory tests (SHAPIRO-ILAN et al., 2000, 2003; DUNCAN et al., 2003; STUART et al., 2004).

The variations in the susceptibility of different stages (larvae and adults) of *H. hampei* can be explained by differences in morphology and behavior. The larvae have a less rigid cuticle, more exposed spiracles, and less mobility, which favors the entrance of the infecting juveniles, unlike the adults of *H. hampe*i that present a more rigid cuticle, small spiracles hidden under the elytra, and large mandibles (BENAVIDES- MACHADO et al., 2010). All these factors contribute to low mortality, making the adult stage of the coffee borer less susceptible to attack by EPNs.

It was possible to observe the complete development of nematodes during the larval stage of *H. hampei* and decomposition of the larvae tissues. Moreover, the typical symptoms of infection by isolates of the genus *Steinernema*, in which the larvae begin to present a yellowish coloration, were observed, as well as typical symptoms of infection by isolates of the genus *Heterorhabditis*, i.e., the infected larvae showed a reddish staining because of the proliferation of bacteria that change the color of the insect (VOSS, 2009). These symptoms of infection were also observed in previous studies (MOLINA; LOPES, 2009; BENAVIDES-MACHADO et al., 2010).

Application of infective juveniles on infested coffee fruit

Both *S. feltiae* and *Heterorhabditis* sp. (IBCB-n 46) applied in aqueous suspension and with xanthan gum were unable to penetrate the fruit through the orifice made by the drill, infect, and cause the death of insects inside the fruit. No IJs were observed in the galleries made by the drills and no insect showed any symptom of infection by the nematodes. However, 5 days after the application of the EPNs, the presence of some IJs was observed on the fruit and these still had some mobility, which was also observed by Molina and López (2009).

Similar tests have been performed previously under laboratory conditions with eight EPNs isolates, five of the genus *Steinernema*, and three of the genus *Heterorhabditis*, and after six days of application none of the isolates caused mortality of the insects inside the fruits, which is in agreement with the observations in the present study (CASTILLO; MARBÁN-MENDOZA, 1996).

Furthermore, Allard and Moore (1989) observed that *Heterorhabditis* sp., applied at a concentration of 240 IJs /per replicate, after 4 days caused 68% mortality in drill adults in the fruit. Based on this previous study, 47 adults and 6 dissected larvae were infected by the nematode, which was not observed in the present study because the insects were alive inside the fruit.

In the study by Molina and López (2009), 7 days after application of the IJs, the parasitism was proportional to the doses applied on the fruit, with the highest mortalities obtained with the highest doses (125 and 625 IJs/per fruit). At

concentrations of 125 and 625 IJs/per fruit, the percentages of mortality caused by *S. feltiae* was 42.88% and 40.58%, and 56.27% and 61.16% by *H. bacteriophora*, respectively.

In tests performed under laboratory conditions, Molina and López (2003) observed that the IJs of *H. bacteriophora* showed high displacement capacity inside the fruit, in contrast with the present study, given that *Heterorhabditis* sp. (IBCBn-46) was not able to move to the interior of the coffee fruit.

Manton et al. (2012) demonstrated that, after 7 days of application, *S. carpocapsae* was able to penetrate the fruits and cause 26.6% mortality in adults and 23.7% mortality in *H. hampei* larvae. According to this study, the *t*-test confirmed that mortality was significantly higher in coffee fruits treated with nematodes.

Heterorhabditis sp. and *Steinernema* sp. were applied at different doses on fallen fruits in the field and, one month after application, the percentages of mortality for *Heterorhabditis* sp. ranged from 53.2% to 82% and for *Steinernema* sp. mortality ranged from 71.25% to 88.19% (LARA et al., 2004).

The doses used in the studies cited above were higher than those used in the present study and this may have provided a higher likelihood of the nematode encountering the coffee berry borer and, therefore, the penetration and infection of insects inside the fruit. Conversely, according to Gaugler et al. (1994) and Selvan et al. (1993), a minimum number of IJs is necessary to "overcome" the immune system of the insect, colonize, and cause its death; however, when this number is exceeded, i.e., when high concentrations of EPNs are used, intraspecific competition may occur that jeopardizes the survival, development, and reproduction of nematodes, reducing their virulence, and consequently their efficiency.

Compatibility of EPNs isolates with cyantraniliprole

Regarding the viability of *S. feltiae* and *Heterorhabditis* sp. (IBCBn-46) after 24 h and 48 h of exposure to the plant protection product, there was a difference between the control and the treatment with cyantraniliprole reducing the viability of IJs in these nematodes, particularly after 48 h of exposure (Table 4).

Although a reduction in viability was observed, the infectivity of *S. feltiae* and *Heterorhabditis* sp. (IBCB-n 46) after being exposed for 24 h to cyantraniliprole was 90% and neither differed from their controls. After 48 h, the infectivity of *S. feltiae* was 86.0%, not differing from the control, whereas the infectivity of *Heterorhabditis* sp. (IBCBn-46) was 76.0%, differing from the control (Table 5). These data suggest that the product had a deleterious effect on the survival of IJs.

Koppenhöfer and Fuzy (2008) observed a negative effect of products belonging to the group of anthranilic diamides, a group to which cyantraniliprole belongs, on the viability of EPNs. The authors found that chlorantraniliprole was compatible with the nematode *H. bacteriophora* when exposed to a tank mix and observed that the combination of the nematode with the product led to high mortality of choroidal larvae, indicating an additive or synergism effect between them.

Table 4. Percentage of viability (M ± SEM) of *Steinernema feltiae* and *Heterorhabditis* sp. (IBCB-n46) after 24 h and 48 h of exposure to the plant protection product cyantraniliprole (protocol IOBC/WPRS), Vainio (1992).

Treatment	Viability[1] (%) 24 hours after exposure	
	Steinernema feltiae (IBCBn-47)	*Heterorhabditis* sp. (IBCBn-46)
Control	80.6 ± 1.5 a*	98.6 ± 0.2 a
Cyantraniliprole	70.4 ± 1.9 b	64.2 ± 2.0 b
CV (%)	3.34	4.28
Treatment	**Viability[1] (%) 48 hours after exposure**	
	Steinernema feltiae (IBCBn-47)	*Heterorhabditis* sp. (IBCBn-46)
Control	68.0 ± 2.5 a	98.2 ± 0.4 a
Cyantraniliprole	55.6 ± 2.1 b	51.4 ± 2.5 b
CV (%)	4.69	4.79

[1]Percentage of alive infective juveniles.
*Means followed by the same letter in the column did not differ among themselves by Tukey's test (p ≤ 5%).

Table 5. Percentage of infectivity (M ± SEM) of *Steinernema feltiae* and *Heterorhabditis* sp. (IBCB-n46) in last instar larvae of *Galleria mellonella* after 24 h and 48 h of exposure to the plant protection product cyantraniliprole (protocol IOBC/WPRS), Vainio (1992).

Treatment (24 hours)	Infectivity (%)[1]	
	Steinernema feltiae (IBCBn-47)	*Heterorhabditis* sp. (IBCBn-46)
Control (IJs + distilled water)	86.0 ± 4.0 a*	96.0 ± 2.4 a
IJs exposed to cyantraniliprole	90.0 ± 3.2 a	90.0 ± 5.5 a
CV (%)	7.19	11.53
Treatment (48 hours)	**Infectivity (%)**	
	Steinernema feltiae (IBCBn-47)	*Heterorhabditis* sp. (IBCBn-46)
Control (IJs + distilled water)	90.0 ± 6.3 a*	96.0 ± 2.4 a
IJs exposed to cyantraniliprole	86.0 ± 7.5 a	76.0 ± 6.3 b
CV (%)	13.45	10.7

[1]Mean number of larvae killed by treatment.
*Means followed by the same letter in the column did not differ among themselves by the Tukey's test (p ≤ 5%).

In a study carried out by Rovesti and Deseö (1990), the compatibility of various phytosanitary products against *H. bacteriophora*, *S. carpocapsae,* and *S. feltiae* was studied and low viability of *H. bacteriophora* was observed after exposure to the fungicides mancozeb and metalaxyl + folpet. However, high infectivity in *G. mellonella* larvae was observed, which is in agreement with the observations in the present study. The infectivity may be high even with low viability because few or only one infecting juvenile can cause the death of an insect.

In contrast, Bortoluzzi et al. (2013) assessed the compatibility of *Heterorhabditis* sp. (IBCB-n 40) with the insecticide carbofuran (Furadan 350 FS®) and observed that the product did not significantly affect the viability of the IJs; however, it caused a 72% reduction in the infectivity of the EPNs. The same observation was reported by Andaló et al. (2004), in which the insecticide carbofuran did not affect the viability but reduced 50% of the infectivity in *S. arenarium* IJs.

Some authors believe that it is this change in infectivity, and not in viability, that is related to the effect of the products on characteristics related to the behavior of the nematode. This could lead to inhibition of movement, dispersion and attraction to the host, and inhibition of reproduction and development, in such a way that even if the nematode is alive, it will not cause death (ROVESTI; DESEÖ, 1990; LAZNIK et al., 2012).

Based on a study by Andaló et al. (2004), some products act as inhibitors of lipid synthesis, which are the main energy sources for the IJs and represent approximately 30% of their body weight, and it is through these reserves that the EPNs remain alive until they find a new host to parasitize. Thus, cyantraniliprole may not affect the behavior or the lipid reserves of IJs of *S. feltiae* (IBCB-n 27) and *Heterorhabditis* sp. (IBCB-n 46).

According to Vainio's protocol (1992), more than 50% reduction in mortality rates or reduction of infectivity in relation to the control are indicative of incompatibility. Therefore, cyantraniliprole can be considered compatible with the nematodes evaluated in the present study because the reduction in viability as well as infectivity was lower than 50%.

The results obtained enable us to conclude that, apart from isolates *S. puertoricense* (CER-n 125) and *Steinernema* sp. (IBCB-n 27), all isolates were pathogenic on adults and larvae of *H. hampei*. *Steinernema feltiae* and *Heterorhabditis* sp. (IBCB-n 46) were not able to infect adults and larvae of *H. hampei* in the interior of the fruit when applied on its surface. The insecticide cyantraniliprole affected the viability but did not affect the infectivity of *S. feltiae* and *Heterorhabditis* sp. (IBCB-n 46).

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