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Foliar application of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) for the control of *Diatraea saccharalis* in greenhouse

Aplicação foliar de nematóides entomopatogênicos (Rhabditida: Steinernematidae e Heterorhabditidae) para o controle de *Diatraea saccharalis* em casa de vegetação

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Abstract

The sugarcane borer, *Diatraea saccharalis*, makes tunnels in the cane stalk, causing weight loss, as well as bud death, impairing germination. The weakened stalks are also more prone to breakage by wind, and in young plants the inner whorl of the leaves can die, resulting in a condition known as "dead heart". Chemical control is used, but with low efficiency. This work was designed to test biological control of *D. saccharalis* using entomopathogenic nematodes (NEPs). Two trials in the greenhouse were performed using *Heterohabditis baujard* LPP7 and *Steinernema carpocapse* NCAll by foliar application associated with adjuvants. In the first assay, the number of holes per stalk was 3.4 for the control without nematodes and with Joint* Oil; 3.14 for the control without nematodes and with Gotafix®; 2.44 for *S. carpocapsae* NCAll + Joint* Oil; 2.06 for *H. baujardi* LPP7 + Gotafix® and also for *S. carpocapsae* NCAll + Gotafix®; and 1.84 for *H. baujardi* LPP7 + Joint* Oil. In the second assay, the treatments showed an average of 4.78 holes per stalk for the control without nematodes and with Gotafix®; 4.76 for the control without nematodes and with Joint* Oil; 2.18 for *S. carpocapsae* NCAll + Joint* Oil and for *S. carpocapsae* NCAll + Gotafix®; 1.96 for *H. baujardi* LPP7 + Gotafix®; and 1.90 for *H. baujardi* LPP7 + Joint* Oil. In the two experiments, the treatments with and without the EPNs differed significantly, but there was no difference between the adjuvants or between the two EPN species.
Key words: Biological control, sugarcane borer, NEPs, *Saccharum officinarum*

Resumo

A broca-da-cana, *Diatraea saccharalis*, causa danos à cana-de-açúcar devido ao seu hábito de formar galerias nos colmos, o que acarreta em perda do peso do produto, redução da sacarose e a seca dos ponteiros. O controle químico é utilizado, porém, com baixa eficiência, pois a larva permanece maior parte do tempo de seu desenvolvimento dentro dos colmos. Este trabalho teve como objetivo testar o controle biológico da *D. saccharalis*, utilizando-se nematóides entomopatogênicos (NEPs). Para tanto, foram realizados dois ensaios em casa de vegetação utilizando-se *Heterohabditis baujardi* LPP7 e *Steinernema carpocapse* NcAll por pulverização foliar, associados a produtos adjuvantes. No primeiro ensaio, o número médio de furos causados pela broca-da-cana, nas testemunhas Joint* Oil e Gotafix® foram de 3,4 e 3,14 respectivamente. Já quanto aos tratamentos, o número de furos por colmo analisado foi de 2,44 para *S. carpocapsae* NCAll + Joint* Oil; 2,06 para *H. baujardi* LPP7 + Gotafix®; 2,06 para *S. carpocapsae* NCAll + Gotafix®; e 1,84 para *H. baujardi* LPP7 + Joint* Oil. Quando o experimento

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foi repetido, o número médio de furos nas testemunhas Gotafix® e Joint* Oil, foram respectivamente 4,78 e 4,76. Quanto aos tratamentos *S. carpocapsae* NCAll + Joint* Oil, *S. carpocapsae* NCAll + Gotafix®, *H. baujardi* LPP7 + Gotafix® e *H. baujardi* LPP7 + Joint* Oil, apresentaram o número médio de 2,18; 2,18; 1,96 e 1,96 furos por colmos avaliados. Assim, ambos nematóides controlaram a broca da cana, contudo não houve diferença significativa entre NEPs e produtos adjuvantes.
Palavras-chave: Controle biológico, broca-da-cana, NEPs, *Saccharum officinarum*

Introduction

The Brazilian sugarcane harvest for 2010-2011 amounted to 624.99 million tonnes (metric tons). Of this quantity, 53.8% (336.2 million tonnes) was used to produce 27.7 billion liters of ethanol and 46.2% (288.7 million tonnes) was used to make sugar (CONAB, 2011). These figures make Brazil among the world leaders in both products. Brazil has been a leading producer of sugar since colonial times, while the importance of ethanol production dates only from the late 1970s, and is becoming more important because of the efforts of many countries to reduce their reliance on petroleum as a fuel source (MOZAMBANI et al., 2006). However, as all crops, sugarcane is subject to attack by pests during its growing cycle, reducing its quality and productivity. These problems are becoming worse as the area planted with cane increases (MACEDO; MACEDO, 2007).

The main sugarcane pest in Brazil is the sugarcane borer, *Diatraea saccharalis* (Fabricius) (Lepidoptera: Crambidae), due to its wide distribution and the damage it causes (GALLO et al., 1988; LIMA FILHO, 1999). The larvae of this moth bore tunnels in the cane stalk, causing weight loss, as well as bud death, impairing germination. The weakened stalks are also more prone to breakage by wind and in young plants the inner whorl of the leaves can die, resulting in a condition known as "dead heart" (GALLO et al., 2002). Each 1% of intensity of infestation by this pest causes weight loss of 0.77%, resulting in losses of 0.25% in sugar output or 0.20% in ethanol yield (GALLO et al., 2002; CAMPOS; MACEDO, 2004).

The use of larvae of the endoparasitoid wasp *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae) to control the sugarcane borer has been shown to be efficient (ALMEIDA; STINGEL, 2005) and is the most widely used method in Brazil (PINTO; GARCIA; OLIVEIRA, 2006). The success in using this species to control the cane borer has led to investments by laboratories in more efficient and profitable mass breeding methods (CARVALHO, 2006).

Chemical control is also employed, but it is not very efficient because the borers remain inside the stalks, protected from contact with the pesticide (PINTO; GARCIA; OLIVEIRA, 2006). The use of entomopathogenic nematodes (EPNs) (Rhabditida: Steinernematidae and Heterorhabditidae) has been successful in many countries to control various crop pests, especially those that traverse the soil or inhabit cryptic environments (LEITE et al., 2006, DOLINSKI; VALLE; STUART, 2006). EPNs of the *Steinernema* and *Heterorhabditis* genera live in the soil and are natural parasites of insects found in this ecosystem, as well as having a symbiotic relationship with bacteria of the *Xenorhabdus* and *Photorhabdus* genera, respectively (FORST; CLARKE, 2002).

Entomopathogenic nematodes have already been tested against other sugarcane pests. Two studies by Spaull (1988, 1990) in Africa showed that the EPNs *Heterorhabditis* and *Steinernema* can reduce the population of *Eldana saccharina* Walker (Lepidoptera: Pyralidae) on sugarcane. Also in Africa, Pillay et al. (2009) obtained 100% mortality of *E. saccharina* by using isolates of these two EPN genera 48 hours after treatment.

In the United States and Japan, billbugs of the *Sphenophorus* genus have been efficiently controlled by the use of *H. bacteriophora* Poinar

and *S. carpocapsae* (Weiser) Wouts, Mracek, Gerdin & Bedding (SMITH, 1994; SHAPIRO-ILAN; GOUGE; KOPPENHÖFER, 2002).

In Brazil, Leite et al. (2002) obtained 80% mortality of the sugarcane spittlebug, *Mahanarva fimbriolata* (Fabr.) (Hemiptera: Cercopidae) by applying *Heterorhabditis* sp. and *Steinernema* sp. nematodes. In turn, Tavares et al. (2007) applied 60 infective juveniles (IJs) per cm² of *H. indica* Poinar, Karunakar & David IBCB-n5 against *Sphenophorus levis* (Coleoptera: Curculionidae) and obtained mortality of 95% in the laboratory and 85% in the greenhouse. When they used *Steinernema* sp. IBCB-n6, the rates were 73% in the laboratory and 42% in the greenhouse.

The entomopathogenic nematodes *Steinernema glaseri* (Steiner) Santa Rosa and *H. indica* IBCB-n5 were tested against eggs and larvae of *Migdolus fryanus* Westwood (Coleoptera: Cerambycidae) in laboratory experiments. There was no significant difference between the treatments. However, in both cases there was penetration by the nematode and reduction of the viability of the infected eggs. Regarding the *M. fryanus* larvae, *S. glaseri* caused 100% mortality and *H. indica* caused 80%, in both cases significantly different from the control (MACHADO et al., 2005).

For leaf application, the most commonly applied species is *S. carpocapse* NCall (BAUR; KAYA; TABASHNIK, 1997; BAUR; KAYA; THURSTON, 1995; PEREZ, et al., 2000). However, there are no reports in the literature on the leaf application of *H. baujardi* LPP7. Therefore, the aim of this study was to test a new alternative for control of *D. saccharalis* in sugarcane, by comparing the application of the entomopathogenic nematodes *S. carpocapse* NCall and *H. baujardi* LPP7 by leaf spraying, with and without adjuvants, in a greenhouse experiment.

Material and Methods

The experiments were performed at the Entomology and Phytopathology Laboratory of Universidade Estadual do Norte Fluminense Darcy Ribeiro (state of Rio de Janeiro, Brazil) and in a greenhouse on the campus.

The two EPN species, *H. baujardi* Phan et al. LPP7 and *S. carpocapae* (Weiser) NCAll, were multiplied in larvae of the last instar of *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) in a breeding group maintained by the laboratory. These larvae were infested in Petri dishes (9 cm diameter) lined with two sheets of filter paper moistened with 2 mL of a nematode suspension containing approximately 200 IJs. The dishes were sealed with plastic film, identified and placed in a BOD chamber at 25 ± 1° C and RH > 80% for 48 hours. The dead larvae were transferred to modified White traps (WHITE, 1927). These traps were made of Petri dishes (9 cm in diameter) with a PVC ring (3 cm diameter x 1 cm height), with a sheet of filter paper placed over the ring (2 cm x 4 cm). The dead larvae were placed over the paper and distilled water was added in the base of the dish until reaching half way up the PVC ring. The traps were then placed in the BOD chamber (25 ± 1 °C and RH > 80%) until emergence of the IJs. These were collected with Pasteur pipettes every other day for 12 days, placed in cell culture bottles (1,000 mL), identified regarding collection date and stored in a climate-controlled chamber at 16 ± 1 °C and RH > 80% for no more than one week before the experiments, so that the juveniles would not lose their infectiveness.

The *D. saccharalis* specimens and sugarcane setts (stalk sections) were donated by Universidade Federal Rural do Rio de Janeiro *campus* Dr. Leonel Miranda (UFRRJ) in Campos dos Goytacazes, RJ

The experimental design was fully randomized blocks, with six treatments and ten repetitions. Besides the IJs of *H. baujardi* LPP7 and *S. carpocapae* NCAll, two chemical adjuvants were also tested, Joint* Oil (Dow AgroSciences) and Gotafix® (Milenia) (Table 1). Two experiments were performed, one after the other, with the same treatments, to confirm the results.

The experiments were conducted with six 20-liter pots, each planted with five sugarcane setts (variety RB 72454). To determine the volume of solution to be applied in each treatment, initial tests were performed using a sprayer with 5 L capacity. Four months after planting, two *D. saccharalis* caterpillars, 12 days old, were placed on each of three axils per stalk, for a total of six caterpillars per stalk and 30 per pot. After 48 hours, each pot was sprayed with 1 L of the respective treatment solution (Table 1).

The evaluation of the mortality of the *D. saccharalis* larvae was carried out one week after application of the EPNs. All the stalks were cut at soil level and then lengthwise. In each treatment the dead larvae were collected and placed in White traps, to prove the efficiency of the EPNs.

The data on the dead larvae for each stalk were submitted to analysis of variance and the means were compared by the Tukey test (P=0.05).

Table 1. Treatments used in each assay under greenhouse conditions.

	Treatments					
Constituents	A	B	C	D	E	F
Number of infective juveniles of *H. baujardi* LPP7	-	-	500.000	500.000	-	-
Number of infective juveniles of *S. carpocapsae* All	-	-	-	-	500.000	500.000
Joint* Oil	50 mL	-	50 mL	-	50 mL	-
Gotafix®	-	5mL	-	5 mL	-	5 mL

Source: Elaboration of the authors.

Results and Discussion

In the two experiments, the treatments with and without the EPNs differed significantly, but there was no difference between the adjuvants or between the two EPN species.

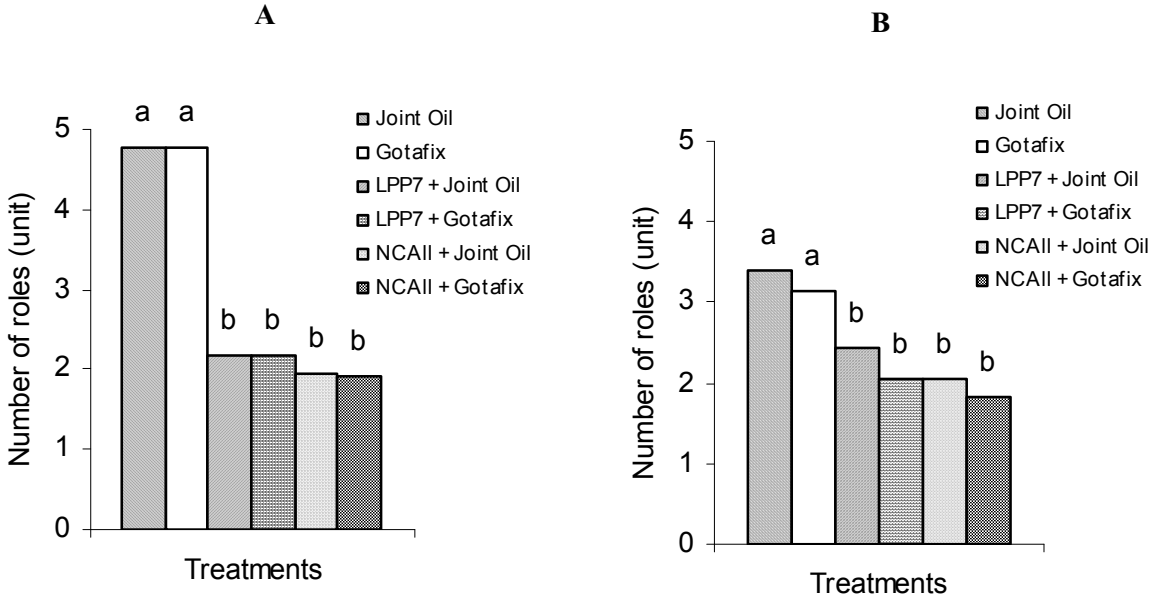
In the first assay, the number of holes per stalk was 3.4 for the control without nematodes and with Joint* Oil; 3.14 for the control without nematodes and with Gotafix®; 2.44 for *S. carpocapsae* NCAll + Joint* Oil; 2.06 for *H. baujardi* LPP7 + Gotafix® and also for *S. carpocapsae* NCAll + Gotafix®; and 1.84 for *H. baujardi* LPP7 + Joint* Oil ($P<0.01$; gl = 5; F = 6.2858) (Figure 1A). In the second assay, the treatments showed an average of 4.78 holes per stalk for the control without nematodes and with Gotafix®; 4.76 for the control without nematodes and with Joint* Oil; 2.18 for *S. carpocapsae* NCAll + Joint* Oil and for *S. carpocapsae* NCAll + Gotafix®; 1.96 for *H. baujardi* LPP7 + Gotafix®; and 1.90 for *H. baujardi* LPP7 + Joint* Oil ($P<0.01$; gl = 5; F = 183.0643) (Figure 1B).

In the treatments with application of EPNs, the number of holes was lower than in the treatments without application of the EPNs. The suspension of EPNs sprayed on the plants ran from the leaves to the inside of the stalk via the leaf sheaths, possibly carrying the nematodes, which reached the larvae inside the stalk. Proof that the death of the larvae found in the leaf sheath regions and inside the stalk of the treated plants was caused by the EPNs was achieved by obtaining IJs from the dead caterpillars, placed in the modified White traps.

There was no difference in the level of control provided by the two species tested, even though they have different foraging strategies. *H. baujardi* is attracted by the cues given off by the target host, such as carbon dioxide, actively searching for the host in a "cruiser" strategy. In contrast, *S. carpocapsae*

has an "ambusher" strategy, whereby it senses the approach of a host and jumps, without directional response, trying to penetrate the host's cuticle when successfully landing on one (ISHIBASHI, 2002; LEWIS, 2002). Lewis (2002) reported that cruiser EPNs are more effective against insects with low motility in the soil while ambushers are more effective against motile targets. However, in this work there were no significant differences between the species, because the form of moving from the leaf to the host was the same. The architecture of the sugarcane leaf and sheath, in the shape of a trough, favors the flow of the aqueous solution containing the EPNs in suspension to the inside the stalk. Therefore, the anatomical characteristic of this plant probably provided the same opportunity for both nematode species to find the holes bored by the host.

Figure 1 A-B. Two bioassays showing number of holes caused by the sugar borer, *Diatraea saccharalis*, in sugarcane setts in different treatments under greenhouse conditions. Means with the same letter are not statistically different by Tukey's test ($P < 0.05$). LPP7: *Heterorhbaditis baujardi* LPP7; NCAll: *Steinernema carpocapsae* NCAll. Joint oil and Gota mix: adjuvants.



Source: Elaboration of the authors.

There also was no significant difference between the two adjuvants used. Silva, Silva and Voss (2008) also used the adjuvant Gotafix® in experiments with EPNs, observing that it provided greater survival of *Heterorhabditis* sp. isolate EPNET 19 (Nematoda: Heterorhabditidae). The use of entomopathogenic nematodes to control agricultural pests is now a reality due to their active capacity to find and control the target pests. Since the two nematodes, with the two adjuvants, applied in a semi-controlled situation, reduced the damages caused by *D. saccharalis* in sugarcane stalks, we recommend combining *H. baujardi* LPP7 with Joint Oil*. Besides having an active cruiser foraging strategy, with the ability to seek out the host in the stalk, this nematode is native to Brazil (DOLINSKI et al., 2008).

Based on the results obtained in the present study, it can be concluded that the EPNs tested have potential for use in the biological control of *D. saccharalis* in sugarcane plantations. However,

further research is necessary about the technical and economic feasibility of using this natural enemy of the sugarcane borer in field conditions.

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