BioControl
https://doi.org/10.1007/s10526-024-10272-3

ORIGINAL ARTICLE

Within-host competition between *Cotesia flavipes* and *Heterorhabditis bacteriophora* in *Diatraea saccharalis*

Telliane Santos Salgueiro Silva · **Patricia da Silva Santos** · **Rayanne Ingrid Silva Vieira** · **Aldomário Santo Negrisoli Junior** · **Elio Cesar Guzzo**

Received: 1 February 2024 / Accepted: 12 June 2024

Abstract The larval parasitoid *Cotesia flavipes* (Cameron, 1891) (Hymenoptera: Braconidae) has been widely used for biological control of the sugarcane borer *Diatraea saccharalis* (Fabricius, 1974) (Lepidoptera: Crambidae) in Brazil. The entomopathogenic nematode (EPN) *Heterorhabditis bacteriophora* Poinar, 1976 (Rhabditida: Heterorhabditidae) also has shown to be a promising agent for controlling the pest. However, in some cases, EPNs can negatively affect non-target organisms, such as parasitoids. Thus, this work evaluated the within-host interaction between *C. flavipes* and *H. bacteriophora*. Caterpillars of *D. saccharalis* were offered to adult females of *C. flavipes* for parasitism and then inoculated with *H. bacteriophora* in intervals of three days until the 12th day. After death, caterpillars were kept for four days and dissected to verify the agent that caused death. We observed an intraguild competition between both control agents. When the EPN was applied until the 12th day after parasitism, it developed and killed the parasitoid, even colonizing its larvae and pupae inside the caterpillar body. On the other hand, when the EPN was applied after 12 days from the parasitism, the parasitoid could develop, but not the EPN. We could conclude that the parasitoid *C. flavipes* and the EPN *H. bacteriophora* are not compatible for controlling *D. saccharalis*. If combined in a single control strategy, it must be considered a safe interval of more than 12 days between applications of each of them.

Keywords Biological control · Sugarcane borer · Parasitoid · Entomopathogenic nematode · Parasitism · Intraguild competition

Introduction

The sugarcane *Saccharum officinarum* L. (Poaceae) is currently cultivated in the tropics worldwide. The main objective of industrial sugarcane processing is to obtain purified sugar and ethanol, which generates a considerable amount of biomass (bagasse) rich in lignocellulose. Economic interest in sugarcane has significantly increased in recent decades due to the worldwide demand for sustainable energy production (Cheavegatti-Gianotto et al. 2011).

The sugarcane borer *Diatraea saccharalis* (Fabricius, 1794) (Lepidoptera: Crambidae) is the most

Handling Editor: Eric Wajnberg.

T. S. S. Silva · E. C. Guzzo (✉)
Universidade Federal de Alagoas - UFAL, Programa de Pós-Graduação em Proteção de Plantas - PPGPP, Rio Largo, Alagoas, Brazil
e-mail: elio.guzzo@embrapa.br

T. S. S. Silva
S/A Usina Coruripe Açúcar e Álcool, Coruripe, Alagoas, Brazil

P. da S. Santos · R. I. S. Vieira · A. S. Negrisoli Junior · E. C. Guzzo
Embrapa Tabuleiros Costeiros, Unidade de Execução de Pesquisa de Rio Largo, Rio Largo, Alagoas, Brazil

important pest for the crop in Brazil, where it is widely distributed throughout the country, and in other parts of the world. Its caterpillars make galleries inside the stalks, causing loss of biomass and sugar production, besides killing buds and facilitating fungal infections that cause juice contamination (Cheavegatti-Gianotto et al. 2011; Pinto and Trujillo 2019; Pinto 2021). Several insecticides are registered for use against *D. saccharalis* in Brazil (AGROFIT 2024) but control has been achieved mainly by inundative releases of the larval parasitoid *Cotesia flavipes* (Cameron, 1891) (Hymenoptera: Braconidae) (Cheavegatti-Gianotto et al. 2011; Pinto and Trujillo 2019; Pinto 2021), which is marketed and also produced in several mill facilities.

Cotesia flavipes is a gregarious larval endoparasitoid of several genera of grass stalk borers of the family Crambidae. Adult *C. flavipes* females seek the host caterpillars inside the stalks using chemical cues and insert their eggs into them. Parasitoid larvae feed inside the host and, when completely developed, leave the caterpillar body to pupate outside, forming masses of cocoons attached to it. The host then dies, and the adult parasitoids leave the cocoons, mate, and restart the cycle (Pinto et al. 2021). It originated in Southeast Asia and Australia but has been introduced worldwide for controlling *Diatraea* spp. (Pinto and Trujillo 2019; Pinto 2021). In Brazil, about 3,000,000 ha of sugarcane are currently under control with *C. flavipes* (Costa-Lima et al. 2019; Pinto and Trujillo 2019), which makes it the most released parasitoid in the country. However, when the control of *D. saccharalis* is done only by *C. flavipes*, at least two releases of the parasitoid are necessary during the harvest (Pinto and Trujillo 2019). The relatively slow reduction of the pest population by *C. flavipes* has encouraged research on its use in association with other biological control agents, such as entomopathogenic fungi and parasitoids (Rossoni et al. 2014; Pinto and Trujillo 2019; Oliveira 2022), which is also in good agreement with the integrated pest management philosophy.

Entomopathogenic nematodes (EPNs) have also been highlighted in the last decades as important biological control agents, mainly for cryptic pests (Andaló et al. 2019; Leite et al. 2019; Dolinski 2020). Infective juveniles (IJs) of EPNs from the genus *Heterorhabditis* actively seek out their hosts and enter their bodies, usually through natural openings such as the mouth, spiracles, and anus (Dowds and Peters 2002; Andaló et al. 2019; Dolinski 2020). Once inside the host, the EPNs migrate to the hemocoel, releasing symbiotic bacteria that produce toxins and kill the host through bacterial septicemia within 24–48 h. Nematodes then feed on the bacteria and decomposed host tissues, producing two–three generations inside the host body, depending on the food availability, and abandon the host cadaver to restart the cycle (Forst and Clarke 2002; Andaló et al. 2019; Dolinski 2020). Some studies have evaluated the control of sugarcane pests with EPNs (Leite et al. 2003; Bellini and Dolinski 2012), and the species *Heterorhabditis baujardi* Phan, Subbotin, Nguyen and Moens, 2003 and *Steinernema carpocapsae* (Weiser, 1955) have shown promising results against *D. saccharalis* under greenhouse conditions (Bellini and Dolinski 2012).

The integrated use of more than one natural enemy against a given pest is desirable. However, it can be deleterious when one species kills the other besides the pest (Pell et al. 2008). Interactions involving some control agents of sugarcane pests have been investigated, such as the interaction of the entomopathogenic fungus *Metarhizium anisopliae* with the EPN *Heterorhabditis bacteriophora* Poinar, 1976 (Acevedo et al. 2007); of the fungi *M. anisopliae* and *Beauveria bassiana* with the parasitoid *C. flavipes* (Rossoni et al. 2014); of *M. anisopliae* and *B. bassiana* with the parasitoids *Palmistichus elaeisis* Delvare and LaSalle, 1993, *Tetrastichus howardi* (Olliff, 1893) and *Trichospilus diatraeae* Cherian and Margabandhu, 1942 (Hymenoptera: Eulophidae) (Rossoni et al. 2016); and of *M. anisopliae* and *B. bassiana* with a granulovirus (Pauli et al. 2018). However, the interaction between *C. flavipes* and EPNs has yet to be studied. Therefore, the present work aimed at investigating the possible competition of the larval parasitoid *C. flavipes* and the EPN *H. bacteriophora* within the host *D. saccharalis*, as well as to determine a safe interval for application of both, allowing their integrated use in biological control programs.

Materials and methods

Insects and EPN obtainment

The sugarcane borer *D. saccharalis* and the parasitoid *C. flavipes* were provided by Fitoagro—Controle

Biológico Ltda., a commercial producer of biological control agents located in Maceió, AL, Brazil. The sugarcane borer was reared on the artificial diet of Hensley and Hammond Jr (1968), and the parasitoid was multiplied on this host according to the methodology described by Pinto (2021). The facility provided 14-day-old *D. saccharalis* caterpillars and one-day-old mated *C. flavipes* adult females, ready to be used in the bioassays.

The EPN *H. bacteriophora* was obtained from the Collection of Entomopathogenic Nematodes of Embrapa Tabuleiros Costeiros, Unidade de Execução de Pesquisa de Rio Largo, located in Rio Largo, AL, Brazil. For bioassays, the nematode was multiplied *in vivo* on third to fifth instar caterpillars of the greater wax moth *Galleria mellonella* (L., 1758) (Lepidoptera: Pyralidae), which were reared on the diet of Guerra (1973).

EPN inoculum preparation

Nematode infective juveniles (IJs) were obtained using the methodology of Woodring and Kaya (1988). The *G. mellonella* caterpillars were inoculated with 2 ml of a suspension containing approximately 200 IJs in Petri dishes lined with a double layer of moistened filter paper. Petri dishes were sealed and kept in a Biological Oxygen Demand (BOD) chamber (25 ± 1 °C; 80 ± 5% RH and total scotophase) during nematode development and reproduction. After 5–7 days from the death of caterpillars, IJs were recovered from the cadavers using White traps (White 1927) and stored in aqueous suspension at 12 °C until its use in the bioassays, which occurred within a maximum of 30 days after emergence. The inoculum was quantified immediately before EPN use. A volume of 25 µl of the IJs suspension was poured into four-well ELISA plates with a micropipette and counted under a stereoscopic microscope. The average of the four wells (500 IJs per ml) was considered as the inoculum concentration. The mobility of IJs was also checked during counting.

Bioassays

To evaluate the possible competition between *C. flavipes* and *H. bacteriophora* within the *D. saccharalis* caterpillar body, we performed two experiments in which we initially inoculated *D. saccharalis* caterpillars with *H. bacteriophora* and then offered them to *C. flavipes* adult females for parasitism after different periods (experiment 1), and *vice versa* (experiment 2). However, when *H. bacteriophora* was inoculated before the parasitism by *C. flavipes*, it did not allow the development of the parasitoid (data not shown). So, such treatments were not considered in the present paper.

In the experiment 2, the treatments were as follows: *D. saccharalis* without parasitism nor inoculation (T1); *D. saccharalis* only parasitized by *C. flavipes* (T2); *D. saccharalis* only inoculated with *H. bacteriophora* (T3); and *D. saccharalis* parasitized by *C. flavipes* and inoculated with *H. bacteriophora* immediately after, after three days, after six days, after nine days, and after 12 days (T4, T5, T6, T7 and T8, respectively). All treatments started with 14-day-old *D. saccharalis* caterpillars. For T1, the caterpillars were put in Petri dishes (five caterpillars per dish) lined with a double layer of moistened filter paper and pieces of the same artificial diet they were reared on and kept in a BOD chamber (25 ± 1 °C; 80 ± 5% RH and total scotophase). In the following treatments (T2 to T8), after inoculation, parasitism, or both, all caterpillars were kept in Petri dishes with diet at the same conditions described for T1. For T2, caterpillars were individually exposed to a one-day-old mated *C. flavipes* adult female for parasitism until stinging visualization. For T3, caterpillars were inoculated with 2 ml of the IJs suspension (500 IJs per ml; 1,000 IJs per dish), applied on the filter paper. From T4 to T8, all caterpillars were exposed to parasitism as described for T2 and then inoculated with IJs as described for T3 at the different periods (immediately after, after thee days, after six days, after nine days, and after 12 days, respectively).

Dishes were checked daily for mortality records. After four days from death, the cadavers were dissected to confirm the presence of the parasitoid, nematode, or both. We considered that if the natural enemy was present it was responsible for the mortality, since this is what happens in the field. In the case that both were present, they were together considered responsible for the mortality. Caterpillars from T1 and T2 were dissected at the end of the experiment. We calculated the mean mortality by parasitoid, mortality by parasitoid with pupal mass formation, mortality by nematode, and mortality by both parasitoid and nematode.

Experimental design and statistical analysis

The experiment followed a completely randomized design, with eight treatments and 20 replications each. A set of five caterpillars was considered as one replication. Data were submitted to ANOVA and mean mortalities were compared to each other by Tukey's test ($P = 0.05$). The statistical software used was GENES (Cruz 2013, 2016).

Results

There was no mortality in the control (T1—*D. saccharalis* caterpillars without *C. flavipes* parasitism nor *H. bacteriophora* inoculation), which significantly differed from all others (Table 1). The total mortality of *D. saccharalis* caterpillars caused solely by *C. flavipes* (T2) or *H. bacteriophora* (T3) differed from the control. When *D. saccharalis* caterpillars were parasitized by *C. flavipes* and inoculated with *H. bacteriophora* at different times (T4 to T8), total mortality was not different from that of the biological control agents solely (T2 and T3), but from the control (T1). Mortality in T5 was statistically different from T8 and T1 but not from the other treatments ($F_{7,\,152} = 54.27$; $P < 0.0001$).

When the presence of the death-causing agent was confirmed, the nematode was shown to be the only responsible for caterpillar mortality in the treatments it was applied solely (T3), applied on the same day of parasitism (T4), and applied in the 3rd day after parasitism (T5) (Table 1). When the nematode was applied on the ninth and the 12th days after parasitism (T7 and T8, respectively), it did not cause caterpillar mortality ($F_{7,\,152} = 300.07$; $P < 0.0001$). Parasitoid and cocoon mass formation were found in the treatment with no nematode inoculation (T2), when parasitism occurred nine days before inoculation (T7), and when parasitism occurred 12 days before inoculation (T8) ($F_{7,\,152} = 37.68$; $P < 0.0001$). Nematode + parasitoid was the main cause of mortality when nematode was applied on the 6th day after parasitism (T6) ($F_{7,\,152} = 94.85$; $P < 0.0001$).

Dissection of dead *D. saccharalis* caterpillars from T6 revealed that *H. bacteriophora* had infected *C. flavipes* larvae (Fig 1a) inside the caterpillar body. In T7, there was parasitoid cocoon mass formation, indicating mortality by parasitoid, but caterpillar cadavers also showed a reddish color, characteristic of infection by EPNs of the genus *Heterorhabditis*. When dissecting parasitoid pupal cocoons, we found that the nematode also infected parasitoid pupae, which could be seen by the same reddish color (Fig 1b).

Table 1 Causes of mortality (mean ± SE) of *D. saccharalis* caterpillars submitted to parasitism by *C. flavipes*, inoculated with *H. bacteriophora*, or both, after different periods (25 ± 1 °C; 80 ± 5% RH and total scotophase)

Treatment[a]	Cause of mortality (%)				
	C. flavipes	*C. flavipes* (cocoon mass)	*C. flavipes* + *H. bacteriophora*	*H. bacteriophora*	Total
T1	0.00 ± 0.00c	0.00 ± 0.00c	0.00 ± 0.00c	0.00 ± 0.00c	0.00 ± 0.00c
T2	36.00 ± 6.09b	52.00 ± 5.51a	0.00 ± 0.00c	0.00 ± 0.00c	88.00 ± 3.04ab
T3	0.00 ± 0.00c	0.00 ± 0.00c	0.00 ± 0.00c	88.00 ± 3.37a	88.00 ± 3.37ab
T4	0.00 ± 0.00c	0.00 ± 0.00c	0.00 ± 0.00c	84.00 ± 3.11ab	84.00 ± 3.11ab
T5	2.00 ± 1.38c	0.00 ± 0.00c	0.00 ± 0.00c	76.00 ± 3.73b	78.00 ± 4.08b
T6	9.00 ± 4.92c	0.00 ± 0.00c	72.00 ± 5.51a	6.00 ± 3.58c	86.00 ± 3.58ab
T7	58.00 ± 6.31a	11.00 ± 4.22bc	16.00 ± 4.72b	0.00 ± 0.00c	85.00 ± 3.20ab
T8	73.00 ± 5.08a	21.00 ± 4.92b	1.00 ± 1.00c	0.00 ± 0.00c	95.00 ± 2.86a

Means followed by different letters in the columns are significantly different by Tukey's test ($P < 0.05$)

[a] No parasitism or inoculation (T1); Parasitism by *C. flavipes* only (T2); Inoculation with *H. bacteriophora* only (T3); Parasitism by *C. flavipes* and inoculation with *H. bacteriophora* in the same day, after three days, after six days, after nine days, and after 12 days (T4, T5, T6, T7 and T8, respectively)

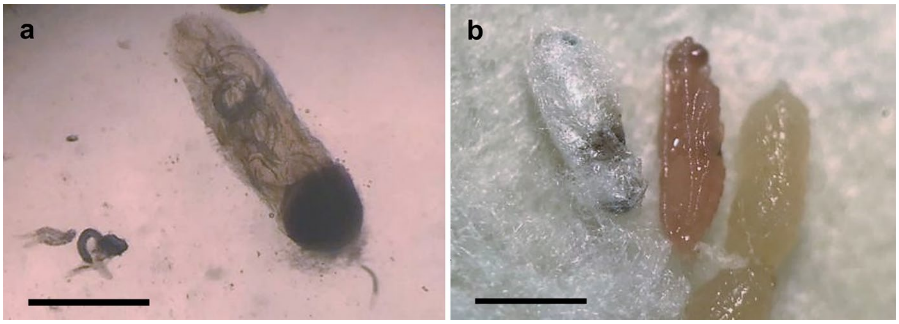

Fig. 1 Entomopathogenic nematode *H. bacteriophora* infecting the larval parasitoid *C. flavipes* in sugarcane borer *D. saccharalis* caterpillars. **a** *H. bacteriophora* infecting *C. flavipes* larvae inside *D. saccharalis* caterpillars when the nematode was inoculated six days after parasitism. **b** *C. flavipes* pupae normal (right), normal within cocoon (left), and with reddish color (center), characteristic of the infection caused by EPNs of the genus *Heterorhabditis*. Scalebars = 2.0 mm

There was an increase in mortality by *C. flavipes* with or without cocoon mass formation and, inversely, a decrease in mortality by *H. bacteriophora* as the time of EPN application after parasitism advanced. The mortality caused by *C. flavipes* + *H. bacteriophora* increased until the 6th day after parasitism when it reached the highest value and decreased again. The highest mortality rate caused by *C. flavipes* was obtained with EPN application on the 12th day after parasitism, and the highest mortality rate by *H. bacteriophora* was obtained when it was applied on the same day of parasitism.

Discussion

Our control was evaluated when the borers were 28 days old, and there was no mortality. At this time, all insects had reached the pupal stage or adulthood and were alive, which is consistent with the mean developmental period of this species in artificial diet (King et al. 1975; Costa et al. 2010; Echeverri-Rubiano et al. 2022). Mortality caused by *C. flavipes* was considered high when applied solely (T2) or nine (T7) and 12 (T8) days before *H. bacteriophora* inoculation. According to Pádua (1983), the age when caterpillars were submitted to parasitism (14 days old) is suitable due to the high fat and water content in its tissues. In all treatments with *H. bacteriophora*, the *D. saccharalis* caterpillars died within two days after inoculation. We did not go forward to inoculation on the 15th day after parasitism because, at this time, *D. saccharalis* caterpillars were already dead, some of them with cocoon mass formation or even with adult parasitoids emerged.

Results from T4 to T8, in which *D. saccharalis* caterpillars were submitted to both *C. flavipes* and *H. bacteriophora*, showed intraguild competition between the parasitoid and the EPN. When *H. bacteriophora* was inoculated at the same time (T4) or on the 3rd day after parasitism by *C. flavipes* (T5), the nematode prevailed and was the only or the main cause of caterpillar mortality, showing that it is more aggressive than the parasitoid. When *H. bacteriophora* was inoculated six days after parasitism by *C. flavipes* (T6), both natural enemies were able to develop and were found together in most of the dead *D. saccharalis* caterpillars.

The infection of *C. flavipes* larvae and pupae by *H. bacteriophora* showed that the EPN has an advantage over the parasitoid, impairing its development and leading it to death. Even being generally considered beneficial organisms, EPNs can eventually impair beneficial insects, such as parasitoids (Akhurst and Smith 2002). After the inundative application of EPNs, there is an impact associated with their non-selectivity, as these organisms are able to infect susceptible non-target species at the time of their application (Atwa et al. 2013). The interference of EPNs from the families Heterorhabditidae and Steinernematidae on the development of endoparasitoid wasps from the families Braconidae and Ichneumonidae has been documented in the literature (Kaya 1978; Kaya and Hotchkin 1981; Shannag and Capinera 2000). Everard et al. (2009) also found that, when the EPN *Heterorhabditis downesi* Stock, Griffin and Burnell was applied on the braconid parasitoid *Bracon hylobii* Ratz. (Hymenoptera: Braconidae) feeding on larvae of the weevil *Hylobius abietis* L. (Coleoptera: Curculionidae), the EPN infected the parasitoid larvae, and there was a reduction in cocoon formation and fewer cocoons eclosed. The EPN belongs to the same genus, and the parasitoid belongs to the same family as those of the present study. However, *B. hylobii* is an ectoparasitoid, so the parasitism occurred outside the host pest body.

On the other hand, when the EPN was inoculated during the late larval stage of the parasitoid, in the ninth and 12th days after parasitism (T7 and T8, respectively), the parasitoid was able to develop within the host's body, being the main cause of caterpillar mortality. Parasitoids may die of starvation when the quality of the host is reduced by nematode infection. Besides this, when the IJs encounter the parasitoid larvae developing inside the host, they can also enter its body like they do with the host pest. However, there seems to be a development threshold beyond which EPN can no longer impair the parasitoid's development. It is possible that when parasitoids are in an advanced developing stage, they have consumed most of the host resources, and the nematode and its symbiotic bacteria cannot reproduce.

It was evident that *C. flavipes* and *H. bacteriophora* interfere with the development of each other within *D. saccharalis*. If the EPN infests the host before, the parasitoid is killed by it. On the other hand, if the parasitism occurs early, the nematode and its symbiotic bacteria do not have enough food to develop. In any case, the combined application results in a waste of resources.

Our results showed that *C. flavipes* development and parasitism in *D. saccharalis* are not impaired if *H. bacteriophora* is applied 12 days after parasitism. This is approximately the same duration of the nematode cycle inside the host (Neves et al. 1998; Andaló et al. 2019). However, our experiment was carried out with a parasitism/inoculation pressure higher than expected in the field. The dose we applied in the laboratory was 500 nematode IJs per ml and the manufacturer's recommended dose for the field results in approximately 114,000 IJs per ml. All IJs applied in the laboratory are potentially able to infect the caterpillars. On the other hand, field application is currently addressed to the planting furrow and the vast majority of the nematodes will not be in contact with the *D. saccharalis* caterpillars. Additionally, under field conditions, EPN pathogenicity is thought to be reduced, mainly in tropical regions, due to the sensitivity to high temperature and other environmental conditions, which affects IJs metabolic processes, such as the rate of nutritional reserves use, mobility, survivorship, and capacity of infection, development, and reproduction (Dunphy and Webster 1986; Kaya and Gaugler 1993; Glazer 2002; Andaló et al. 2019; Dolinski 2020), and also to natural enemies and other antagonists (Kaya 2002). Furthermore, the IJs that abandon the host cadaver would be inside the borers galleries in the sugarcane stalk and would hardly find another caterpillar infested by *C. flavipes*. Consequently, the adult parasitoids that emerged from the cocoons would hardly find another caterpillar already infected by *H. bacteriophora*. Thinking of a strategy that both biocontrol agents could be used in order that one will kill the insects that escaped from the other, the safe interval for both is more than 12 days, independent of which would be applied before, because at this time substantial number of wasps may still be active in the field with potentially detrimental effects.

Although there are no products based on EPNs registered for the control of *D. saccharalis* in Brazil, biopesticides based on *H. bacteriophora* were recently launched, registered for the control of several pests, including the weevil *Sphenophorus levis* (Vaurie, 1978) (Coleoptera: Curculionidae) in sugarcane (AGROFIT 2024). Thus, the present study brings essential initial information regarding pest management in this crop since both biological control agents studied here may be present simultaneously in the sugarcane fields. Knowing their interaction is an essential condition for the definition of control strategies for the target pests. However, it is still necessary to understand the dynamics of the interactions under field conditions in order to validate the best strategy for applying these biocontrol agents in the sugarcane crop.

In conclusion, we have found that there is intraguild competition between the parasitoid *C. flavipes* and the EPN *H. bacteriophora* in the parasitism of *D. saccharalis*. *Heterorhabditis bacteriophora* is able to develop inside the host only if it is inoculated until six days after the parasitism by *C. flavipes*, and *C. flavipes* can develop inside the host only if *H. bacteriophora* is inoculated from nine days after parasitism.

The safe interval between applications is more than 12 days for integrating both biocontrol agents.

Acknowledgements The authors are grateful to Fitoagro Controle Biológico Ltda (Maceió, AL, Brazil) for providing the insects and the artificial diet used in bioassays and to Dr. João Gomes da Costa, Embrapa Alimentos e Territórios (Maceió, AL, Brazil), for helping us with the statistical analysis.

Author contributions TSSS: Methodology, validation, investigation, data curation, formal analysis, writing–original draft, writing–review and editing. PSS: Investigation, data curation, writing–review and editing. RISV: Investigation, data curation, writing–review and editing. ASNJ: Conceptualization, resources, methodology, validation, data curation, formal analysis, supervision, writing–review and editing. ECG: Conceptualization, resources, methodology, validation, data curation, formal analysis, supervision, writing–original draft, writing–review and editing.

Funding This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—Brasil (CAPES)—Finance Code 001 and by Fundação de Amparo à Pesquisa do Estado de Alagoas—Brasil (FAPEAL)—Process 60030.001243/2018.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This research did not involve any studies with human participants or animals (vertebrates).

Consent for publication All authors consent to publication.

References

Acevedo JPM, Samuels RI, Machado IR, Dolinski C (2007) Interactions between isolates of the entomopathogenic fungus *Metarhizium anisopliae* and the entomopathogenic nematode *Heterorhabditis bacteriophora* JPM4 during infection of the sugar cane borer *Diatraea saccharalis* (Lepidoptera: Pyralidae). J Invert Pathol 96:187–192

AGROFIT (2024) Sistema de Agrotóxicos Fitossanitários. Ministério da Agricultura e Pecuária, Brasil. http://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons. Accessed 17 January 2024

Akhurst R, Smith K (2002) Regulation and safety. In: Gaugler R (ed) Entomopathogenic nematology. CABI, New York, pp 311–332

Andaló V, Acevedo JPM, Negrisoli Júnior AS, Dalbon VA (2019) Entomopathogenic nematodes. In: Souza B, Vázquez LL, Marucci RC (eds) Natural enemies of insect pests in neotropical agroecosystems. Biological control and functional biodiversity. Springer, Cham, pp 111–122

Atwa AA, Hegazi EM, Khafagi WE, El-Aziz GMA (2013) Interaction of the koinobiont parasitoid *Microplitis rufiventris* of the cotton leafworm, *Spodoptera littoralis*, with two entomopathogenic rhabditids, *Heterorhabditis bacteriophora* and *Steinernema carpocapsae*. J Insect Sci 13:84

Bellini LL, Dolinski C (2012) Foliar application of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) for the control of *Diatraea saccharalis* in greenhouse. Semina: Ciências Agrárias 33(3):997–1004

Cheavegatti-Gianotto A, Abreu HMC, Arruda P, Bespalhok Filho JC, Burnquist WL, Creste S, di Ciero L, Ferro JÁ, Figueira AVO, Filgueiras TS, Grossi-de-Sá MF, Guzzo EC, Hoffmann HP, Landell MGA, Macedo N, Matsuoka S, Reinach FC, Romano E, Silva WJ, Silva Filho MC, Ulian EC (2011) Sugarcane (*Saccharum* X *officinarum*): a reference study for the regulation of genetically modified cultivars in Brazil. Trop Plant Biol 4:62–89

Costa DM, Francez ACC, Rigolin-Sá O (2010) Biologia da broca da cana-de-açúcar (*Diatraea saccharalis*) (Lepidoptera: Crambidae) em dieta artificial. Ciência Et Praxis 3:13–16

Costa-Lima TC, Coelho A Jr, Diniz AJF, Sampaio MV (2019) Parasitoids insects. In: Souza B, Vázquez LL, Marucci RC (eds) Natural enemies of insect pests in neotropical agroecosystems. Biological control and functional biodiversity. Springer, Cham, pp 199–211

Cruz CD (2013) Genes—a software package for analysis in experimental statistics and quantitative genetics. Acta Sci Agron 35:271–276

Cruz CD (2016) Genes software-extended and integrated with the R, Matlab and Selegen. Acta Sci Agron 38:547–552

Dolinski CM (2020) Controle de artrópodes-praga com nematoides entomopatogênicos. In: Fontes EMG, Valadares-Inglis MC (eds) Controle biológico de pragas da agricultura. Embrapa, Brasília, pp 275–288

Dowds BCA, Peters A (2002) Virulence mechanisms. In: Gaugler R (ed) Entomopathogenic nematology. CABI, Wallingford, pp 79–98

Dunphy GB, Webster JM (1986) Temperature effects on the growth and virulence of *Steinernema feltiae* strains and *Heterorhabditis heliothidis*. J Nematol 18:270–272

Echeverri-Rubiano C, Chica-Ramírez HA, Vargas G (2022) Biology of the most widely distributed sugarcane stem borers, *Diatraea* spp. (Lepidoptera: Crambidae), in Colombia. Neotrop Entomol 51:877–885

Everard A, Griffin CT, Dillon AB (2009) Competition and intraguild predation between the braconid parasitoid *Bracon hylobii* and the entomopathogenic nematode *Heterorhabditis downesi*, natural enemies of the large pine weevil, *Hylobius abietis*. Bul Entomol Res 99:151–161

Forst S, Clarke D (2002) Bacteria-nematode symbiosis. In: Gaugler R (ed) Entomopathogenic nematology. CABI, Wallingford, pp 57–77

Glazer I (2002) Survival biology. In: Gaugler R (ed) Entomopathogenic nematology. CABI, Wallingford, pp 169–187

Guerra MS (1973) Bionomia das traças da cera *Galleria mellonella* L. e *Achroia grisella* F. (Lepidoptera – Galleriidae) no Município de Piracicaba, São Paulo. Dissertation, Universidade de São Paulo

Hensley SD, Hammond AM Jr (1968) Laboratory techniques for rearing the sugarcane borer on an artificial diet. J Econ Entomol 61:1742–1743

Kaya HK (1978) Infectivity of *Neoaplectana carpocapsae* and *Heterorhabditis heliothidis* to pupae of the parasite *Apanteles militaris*. J Nematol 10:241–244

Kaya HK (2002) Natural enemies and other antagonists. In: Gaugler R (ed) Entomopathogenic nematology. CABI, Wallingford, pp 189–203

Kaya HK, Gaugler R (1993) Entomopathogenic nematodes. Annu Rev Entomol 38:181–206

Kaya HK, Hotchkin PG (1981) The nematode *Neoaplectana carpocapsae* Weiser and its effect on selected ichneumonid and braconid parasites. Environ Entomol 10:474–478

King EG, Brewer FD, Martin DF (1975) Development of *Diatraea saccharalis* [Lep.: Pyralidae] at constant temperatures. Entomophaga 20:301–306

Leite LG, Machado LA, Aguillera MM, Rodrigues RCD, Negrisoli AS Jr (2003) Patogenicidade de *Steinernema* spp. e *Heterorhabditis* sp. (Nematoda: Rhabditida) à ninfas da cigarrinha da raíz da cana-de-açúcar, (*Mahanarva fimbriolata*). Rev Agricult 78:139–148

Leite LG, Almeida JEM, Chacon-Orozco JG, Delgado CY (2019) Entomopathogenic nematodes. In: Souza B, Vázquez LL, Marucci RC (eds) Natural enemies of insect pests in neotropical agroecosystems. Biological control and functional biodiversity. Springer, Cham, pp 213–221

Neves PJ, Alves SB, Aguillera MM (1998) Produção de nematoides entomopatogênicos. In: Alves SB (ed) Controle microbiano de insetos, 2nd edn. FEALQ, Piracicaba, pp 889–905

Oliveira JS (2022) Associação de *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae) e *Tetrastichus howardi* (Olliff, 1893) (Hymenoptera: Eulophidae) no controle de *Diatraea saccharalis* (Fabricius, 1794) (Lepidoptera: Crambidae). Dissertation, Universidade de São Paulo

Pádua LEM (1983) Biologia comparada de *Apanteles flavipes* (Cameron, 1891) (Hymenoptera: Braconidae) para determinação das exigências térmicas. Dissertation, Universidade de São Paulo

Pauli G, Mascarin GM, Eilenberg J, Delalibera Júnior I (2018) Within-host competition between two entomopathogenic fungi and a granulovirus in *Diatraea saccharalis* (Lepidoptera: Crambidae). Insects 9:64

Pell JK, Baverstock J, Roy HE, Ware RL, Majerus MEN (2008) Intraguild predation involving *Harmonia axyridis*: a review of current knowledge and future perspectives. BioControl 53:147–168

Pinto AS, Trujillo SEL (2019) Sugarcane. In: Souza B, Vázquez LL, Marucci RC (eds) Natural enemies of insect pests in neotropical agroecosystems. Biological control and functional biodiversity. Springer, Cham, pp 413–425

Pinto AS (2021) O caso de *Cotesia flavipes* na cana-de-açúcar no Brasil. In: Parra JRP, Pinto AS, Nava DE, Oliveira RC, Diniz AJF (eds) Controle biológico com parasitoides e predadores na agricultura brasileira. FEALQ, Piracicaba, pp 159–202

Rossoni C, Kassab SO, Loureiro ES, Pereira FF, Costa DP, Barbosa RH, Zanuncio JC (2014) *Metarhizium anisopliae* and *Beauveria bassiana* (Hypocreales: Clavicipitaceae) are compatible with *Cotesia flavipes* (Hymenoptera: Braconidae). Fla Entomol 97:1794–1804

Rossoni C, Pereira FF, Kassab SO, Rodrigues A, Barbosa RH, Zanuncio JC (2016) Development of Eulophidae (Hymenoptera) parasitoids in *Diatraea saccharalis* (Lepidoptera: Crambidae) pupae exposed to entomopathogenic fungi. Can Entomol 148:716–723

Shannag HK, Capinera JL (2000) Interference of *Steinernema carpocapsae* (Nematoda: Steinernematidae) with *Cardiochiles diaphaniae* (Hymenoptera: Braconidae), a parasitoid of melonworm and pickleworm (Lepidoptera: Pyralidae). Environ Entomol 29:612–617

White GF (1927) A method for obtaining infective nematode larvae from cultures. Science 66:302–303

Woodring JL, Kaya HK (1988) Steinernematidae and Heterorhabditidae nematodes: a handbook of techniques. Arkansas Agricultural Experiment Station, Fayetteville

Telliane Santos Salgueiro Silva has a Master's degree in energy from Biomass and Plant Protection. She is currently working at Coruripe Sugar and Alcohol, Brazil, focusing on agrometeorology, irrigation data analysis, soil and water management, and dam monitoring.

Patricia da Silva Santos is a PhD student in plant production at the Federal University of Alagoas, Brazil. She has worked on seedling production, and her research interests include micrografting.

Rayanne Ingrid Silva Vieira is graduated in agronomy and has worked on improving artificial diets for insect rearing.

Aldomário Santo Negrisoli Junior is an applied entomologist at Embrapa Coastal Tablelands, Brazil. His research focuses on pest management, with an emphasis on the use of entomopathogenic nematodes.

Elio Cesar Guzzo is a researcher of applied entomology at Embrapa Coastal Tablelands and a professor of entomology in the Plant Protection Post-Graduation Program of the Federal University of Alagoas; Brazil. His research focuses on pest management in crops of economic importance for the Brazilian tablelands.