Colegio de Postgraduados

Virulence of three *Heterorhabditis* isolates against the sugarcane weevil *Sphenophorus incurrens* Gyllenhal

Tapia-Alejo, S.[1]; **Grifaldo-Alcántara, P.F.**[2*]; **Palomares-Pérez, M.**[3]; **Acuña-Soto, J.A.**[4]; **Hueso-Guerrero, E.J.**[2]; **Vicente-Pérez, R.**[2]; **Guzmán-Martínez, M.**[5]

[1] Escuela Preparatoria Regional de El Grullo, Avenida Circunvalación Poniente y Carretera al Ingenio S/N, C.P. 48740, El Grullo, Jalisco, México.
[2] Universidad de Guadalajara, Centro Universitario de la Costa Sur, Departamento de Producción Agrícola. Av. Independencia Nacional # 151, Autlán de Navarro, Jalisco, C.P. 48900, México.
[3] Centro Nacional de Referencia de Control Biológico SENASICA-DGSV, Km 1.5 Carretera Tecomán-Estación FFCC, Colonia Tepeyac, C.P. 28110 Tecomán, Colima, México.
[4] Instituto Tecnológico Superior de Tlatlauquitepec, Carretera Federal AmozocNautla Km. 122+600 Almoloni Tlatlauquitepec, Pue., Teziutlán-Acajete, C.P. 73907 Puebla, México.
[5] Universidad Autónoma de Guerrero, Facultad de Matemáticas, C.P. 39087, Chilpancingo, Guerrero, México.
* Correspondence: fabian.grifaldo@academicos.udg.mx

ABSTRACT

Objective: To evaluate the effect of three *Heterorhabditis* nematode isolates obtained from different agroecosystems on adults of *Sphenophorus incurrens*, a phytosanitary problem in sugarcane (*Saccharum* spp.) cultivation.

Design/Methodology/Approach: Adult weevils were collected from the field using bait traps and transported to the laboratory. To assess LC_{50}, LT_{50}, and the efficacy of these native nematodes, bioassays were conducted using doses of 0, 10, 50, 100, 200, 500, 1000, and 5000 infective juveniles (IJs)/adult weevil in 12-well cell culture plates over a period of 10 days.

Results: All three isolates exhibited pathogenicity against sugarcane weevils at all tested doses, except the control. The most virulent strain was *Heterorhabditis bacteriophora* (C-23), isolated from cattle-grazed soils, with an LC_{50} of 2,626 IJs/weevil ($P<0.0001$) and an LT_{50} of 17.7 days ($P<0.0001$).

Limitations/Implications: This is the first known study evaluating EPNs against adult *S. incurrens* under laboratory conditions. Future studies should include field evaluations of these nematodes and their target pest.

Findings/Conclusions: The observed variation in weevil mortality likely relates to the ecological niche, host interaction, and life history of each nematode isolate. These findings support the potential of entomopathogenic nematodes as a viable biological control option for managing *S. incurrens* in sugarcane cultivation.

Keywords: Biological control, bioassays, Heterorhabditidae, pathogenicity, pest.

Citation: Tapia-Alejo, S., 0Grifaldo-Alcántara, P.F., Palomares-Pérez, M., Acuña-Soto, J.A., Hueso-Guerrero, E.J., Vicente-Pérez, R., & Guzmán-Martínez, M. (2025). Virulence of three Heterorhabditis isolates against the sugarcane weevil *Sphenophorus incurrens* Gyllenhal. *Agro Productividad*. https://doi.org/10.32854/8g1qcb45

Academic Editor: Jorge Cadena Iñiguez
Associate Editor: Dra. Lucero del Mar Ruiz Posadas
Guest Editor: Daniel Alejandro Cadena Zamudio

Received: October 14, 2025.
Accepted: December 26, 2025.
Published on-line: February 18, 2026.

Agro Productividad, 18(12). December. 2025. pp: 197-207.

INTRODUCTION

Sugarcane (*Saccharum* spp.) is considered a crop of significant importance due to the wide range of products derived from it (Santiago-Zárate *et al.*, 2022). In Mexico, the states of Veracruz and Jalisco are the primary contributors, accounting for approximately 50.6%

of the country's sugar production (CONADESUCA, 2024). Like any crop, sugarcane is subject to various phytosanitary issues, including diseases and pests, which negatively impact yield. The main harmful agents include insects, rodents, bacteria, and viruses (Ferrer and Salas, 2024; Rodríguez del Bosque *et al.*, 2014).

Among the insect pest problems affecting sugarcane are termites (*Heterotermes* spp.; Isoptera: Rhinotermitidae), stalk borers (*Diatraea* spp.; Lepidoptera: Crambidae), spittlebugs (*Aeneolamia* and *Prosapia* spp.; Hemiptera: Cercopidae), the white grub complex (*Phyllophaga* spp.; Coleoptera: Scarabaeidae) (Salgado-García *et al.*, 2019), and the sugarcane weevil (*Sphenophorus incurrens* Gyllenhal; Coleoptera: Curculionidae) (Domínguez-Monge *et al.*, 2017; Grifaldo-Alcántara *et al.*, 2023).

In Mexico, the most frequently reported and economically significant weevil species in this agroecosystem is *Sphenophorus incurrens* (Segura-León *et al.*, 2013), a pest responsible for losses of up to 20-30 t ha^{-1} (Barreto-Triana *et al.*, 2014). It has been reported in the states of Veracruz, Oaxaca, Puebla, Michoacán, San Luis Potosí, and Morelos (Sánchez-Bolón, 2017), as well as in Jalisco, where its presence was first detected in 2020 (Grifaldo-Alcántara *et al.*, 2023). This pest damages sugarcane stands by creating patchy areas in the crop. However, the larvae are the primary agents of injury, developing in the underground portion of the stalk, penetrating the base, and creating galleries in various directions. Their feeding facilitates the entry of phytopathogenic bacteria, leading to root rot and wilting of both primary and secondary roots (Grifaldo-Alcántara *et al.*, 2023), including *Colletotrichum falcatum*, *Fusarium* spp., and *Nigrospora* spp. (Joyce *et al.*, 2016). These pathogens reduce sugar quality and can result in yield losses of 20-30 t ha^{-1} (Barreto-Triana *et al.*, 2014).

According to their biology, female weevils are polyandrous and may mate multiple times with the same or different males, which enhances reproductive success and can increase fertility and fecundity by up to 70% (Arnqvist *et al.*, 2004). Various developmental stages of the weevil can be found inside sugarcane stools following field burning and harvest. Within the stalks, pupae continue to develop and move toward the roots until reaching adulthood (Segura-León *et al.*, 2014). As adults, they are attracted to the regrowing canes (ratoon crops) after cutting. Females lay eggs individually near the base of the plant, and upon hatching, the early larval stages feed on plant tissue and bore into the stalks, creating galleries (Segura-León *et al.*, 2013).

The primary strategy for controlling this pest relies on the use of chemical products, which are costly, environmentally harmful, and pose risks to food safety and human health (Ramírez-Mora *et al.*, 2018). Commonly used chemicals include Endosulfan (Counter 5G®), Bifenthrin (Brigadier 5G®), Fipronil®, and Thiamethoxam® (CICOPLAFEST, 2022). However, chemical applications have shown limited success against the weevil due to the cryptic behavior of the larvae, which reside inside the plant's stalks and roots (Cerda *et al.*, 1999).

In the search for alternative methods, biological control is widely considered a viable strategy for reducing pests in agricultural settings. It offers multiple benefits, including improved farmer economics, environmental protection, and safeguarding producer health (Arredondo-Bernal and Rodríguez del Bosque, 2008). Parasitoids are among

the most commonly used organisms in biological control, alongside predators and entomopathogenic fungi (Zelaya-Molina *et al.*, 2022). However, other microorganisms, such as entomopathogenic nematodes (EPNs), also hold great potential for controlling agricultural and livestock pests (Pacheco *et al.*, 2019). EPNs exhibit strong adaptability to new environments, effective mobility in soil, and active host-seeking behavior, without harming the environment or human health (San-Blas *et al.*, 2019).

The aim of this study was to quantify the *in vitro* pathogenicity of three native strains of entomopathogenic nematodes against the sugarcane weevil *Sphenophorus incurrens* Gyllenhal.

MATERIALS AND METHODS

Insect collection

In sugarcane fields with reported damage from this pest, traps were set following the methodology implemented by the Atencingo Sugar Mill in Puebla, Mexico (personal communication). A total of 250 traps were placed across a one hectare area. The attractant bait consisted of ground sugarcane and molasses diluted in water at a 20% ratio (5:1), which was allowed to ferment beforehand. The baits were placed on the soil near the base of the sugarcane plants. After 24 hours, the traps were checked, and adult weevils were collected using entomological forceps and stored in airtight 250 mL plastic containers. The specimens were then transported to the laboratory, where they were kept in quarantine prior to experimentation.

Entomopathogenic nematodes

Three native isolates from different agroecosystems in the state of Jalisco were evaluated (Table 1). These isolates were obtained using the insect baiting technique with *Galleria mellonella* L. (Lepidoptera: Pyralidae) (Sturhan and Mráček, 2000).

Multiplication EPNs

Each isolate was individually evaluated using third and fourth instar larvae of *Galleria mellonella* (Linnaeus) (Lepidoptera: Pyralidae). The larvae were placed in 60 mm plastic Petri dishes, each containing two circles of Whatman No. 1 filter paper. In each dish, 1.5 mL of water containing infective juveniles (100 IJs per larva) was added (Stock and Goodrich-Blair, 2012), and the dishes were immediately sealed. After 11 days, the larval cadavers were transferred to White traps (White, 1927) to harvest infective juveniles from each isolate.

Table 1. Native entomopathogenic nematode isolates from the state of Jalisco, Mexico.

Agroecosystem	Municipality	State	Latitud	Longitude	Altitude (m)	Species
Cucumber crop	Autlán de Navarro	Jalisco, Méx.	104° 29’	19° 77’	885	*Heterorhabditis bacteriophora* (AUME-20)
Cattle corrals	El Limón	Jalisco, Méx.	104° 03’	19° 47’	804	*Heterorhabditis bacteriophora* (C-23)
Sugarcane crop	Autlán de Navarro	Jalisco, Méx.	19° 45’	104° 19’	901	*Heterorhabditis bacteriophora* (JAAN-19)

Bioassays

Experiments were conducted under laboratory conditions to quantify the pathogenicity of three EPN strains and determine the median lethal concentration (LC_{50}) and median lethal time (LT_{50}) against adult sugarcane weevils (*Sphenophorus incurrens*). Each experiment used 12 well cell culture plates (Biologix®), with each well containing two circles of Whatman No. 1 filter paper, one randomly selected adult weevil, and a piece of sugarcane as food. Treatments consisted of 10, 50, 100, 200, 500, 1000, and 5000 infective juveniles (IJs), plus a control (water=0 IJs), with three replicates conducted at different times. Volumetric counts were performed for each dose applied (Stock and Goodrich-Blair, 2012).

The boxes containing each treatment and nematode strain were maintained at a temperature of 25±2 °C with a 12:12 h light/dark photoperiod. Evaluations were conducted every 24 hours for 10 days after the experiments began. A Probit regression model was used for the experimental design, and data were analyzed using the SAS statistical software.

RESULTS AND DISCUSSION

Based on the results obtained from the evaluation of three Heterorhabditis EPN isolates against adult sugarcane weevils (*Sphenophorus incurrens*), all isolates demonstrated pathogenic effects on the pest. However, *S. incurrens* adults were most susceptible to the *Heterorhabditis bacteriophora* (C-23) isolate, which was the most virulent, with an LC_{50} of 2,626 IJs/weevil (lower limit: 1,084 IJs/weevil; upper limit: 15,288 IJs/weevil; $P<0.0001$). This was followed by *H. bacteriophora* (AUME-20) with an LC_{50} of 69,167 IJs/weevil (lower limit: 7,394; upper limit: 122,675; $P<0.001$), and *H. bacteriophora* (JAAN-19) with an LC_{50} of 11,558 IJs/weevil (lower limit: 3,540; upper limit: 192,990; $P<0.0001$) (Table 2).

According to the estimated mortality curves for *Sphenophorus incurrens* (Figure 1) and the corresponding lower and upper confidence limits across different EPN IJ concentrations, pathogenicity varied among isolates. The most effective isolate was *Heterorhabditis bacteriophora* (C-23), which caused 50% mortality at 2,626 IJs/weevil. This was followed by *H. bacteriophora* (JAAN-19), with 50% mortality at 11,558 IJs/weevil, and *H. bacteriophora* (AUME-20), which required 69,167 IJs/weevil to reach the same mortality rate. As shown in Figure 1, increasing the IJ concentration of all three native isolates led to a corresponding increase in adult *S. incurrens* mortality.

Regarding the median lethal time (LT_{50}; 95% CI) of the three EPN isolates against *Sphenophorus incurrens* based on Probit analysis, the most effective isolate was *H. bacteriophora* (C-23), with an LT_{50} of 17.8 days ($P<0.0001$), a lower limit of 14.8 days, and an upper limit of 22.8 days. This was followed by *H. bacteriophora* (AUME-20), with an LT_{50} of 26 days

Table 2. Median lethal concentration (LC_{50}) of three native EPN isolates.

EPN Isolate	LC_{50} (IJs / weevil)	IC_{DL50} 95% (EPN IJs)		Intercept ± EE	Log10 (Dose) ± EE	P value
		Lower	Upper			
H. bacteriophora (AUME-20)	69167	7394	122675	-3.1±0.6	0.63±0.2	<0.001
H. bacteriophora (C-23)	2626	1084	15288	-3.0±0.5	0.87±0.2	<0.0001
H. bacteriophora (JAAN-19)	11558	3540	192990	-3.9±0.6	0.96±0.22	<0.0001

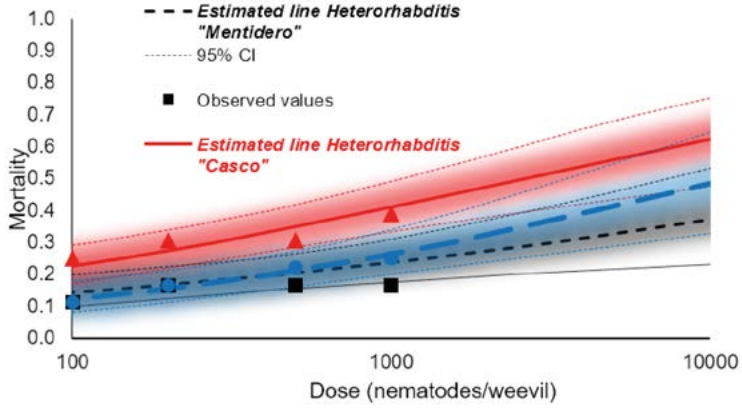

Figure 1. LC_{50} of three *Heterorhabditis bacteriophora* strains (C-23, JAAN-19, and AUME-20) with 95% confidence intervals (CI).

(P<0.0001; CI: 20.1-38.1 days), and *H. bacteriophora* (JAAN-19), with an LT_{50} of 28.2 days (P<0.0001; CI: 22.3-43.0 days) (Table 3).

Figure 2 shows the 95% confidence intervals for the estimated mortality curves and observed mortality values of adult *Sphenophorus incurrens* over time (days). The isolate *Heterorhabditis bacteriophora* (C-23) showed the highest efficacy, causing 50% mortality in 17.8 days. This was followed by *H. bacteriophora* (JAAN-19), with 50% mortality in 26 days, and *H. bacteriophora* (AUME-20), which exhibited a similar effect with 50% mortality in 28.2 days.

Table 3. Median lethal concentration (LC_{50}) of three native EPN isolates.

EPN Isolate	LT_{50} (days)	IC_{DL50} 95% (EPN IJs)		Intercept ± EE	Log10(Dose) ± EE	P value
		Lower	Upper			
H. bacteriophora (AUME-20)	26	20.1	38.1	−4.3±0.3	3.0±0.3	<.0001
H. bacteriophora (C-23)	17.8	14.8	22.8	−3.2±0.2	2.6±0.2	<.0001
H. bacteriophora (JAAN-19)	28.2	21.3	43	−4.0±0.2	2.8±0.23	<.0001

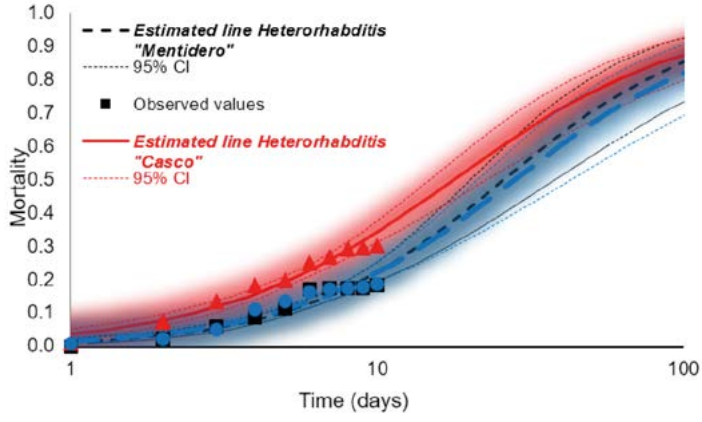

Figure 2. LT_{50} of three native *Heterorhabditis bacteriophora* isolates (C-23, JAAN-19, and AUME-20).

At the end of each experiment, to confirm that the death of *S. incurrens* adults was caused by EPNs, the cadavers were placed in new Petri dishes (100×15 mm) containing a circle of Whatman No. 1 filter paper and 1.5 mL of sterilized water. In all cases, EPN emergence from each isolate was observed.

Therefore, we conclude that all three EPN isolates evaluated against adult *S. incurrens* were effective. However, the isolate *H. bacteriophora* (C-23), collected from cattle farming areas, showed the highest pathogenicity with an LC_{50} of 2,626 IJs/weevil ($P<0.0001$). This variation in pathogenicity among isolates may be attributed to interactions with different pest insects present in the environments from which each isolate was obtained. According to Rodríguez *et al.* (2009), although satisfactory mortality rates were observed in *Galleria mellonella* and *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) using six native *Heterorhabditis* isolates, only *Heterorhabditis* sp. CIA-NE-07 showed higher effectiveness in laboratory conditions, achieving 50% mortality (LC_{50}=625 IJs/larva in 30 days) against six out of ten larvae of *Phyllophaga elenans* Saylor (Coleoptera: Melolonthidae). Similarly, in our study, higher mortality was expected from the *H. bacteriophora* (JAAN-19) isolate, which was collected from sugarcane fields. However, we believe it is possible that *S. incurrens* and this isolate may not have interacted in the field, as the main pest problem in the collection area was the sugarcane borer *Diatraea saccharalis* (personal communication).

It is known that adult sugarcane weevils are sensitive to movement and remain motionless when the soil is disturbed a behavior thought to be an escape mechanism from predators (Segura-León *et al.*, 2014). Their damage appears in patches and they exhibit gregarious behavior within the crop, seeking dark, moist areas such as inside stalks, leaf axils, and roots, where larvae, pupae, and adults coexist and feed in groups (Glibin *et al.*, 2000; Girón-Pérez *et al.*, 2009). Based on this behavior, the use of entomopathogenic nematodes (EPNs) is considered a promising strategy for managing this pest in sugarcane agroecosystems. These nematodes can move through soil pores in search of hosts, being attracted by insect emitted volatiles (Barbercheck and Kaya, 1991; Sáenz-Aponte and Olivares, 2008). According to the results of this study, *Heterorhabditis bacteriophora* (C-23) could effectively regulate both adult and immature stages of the pest, given their coexistence in the same habitat. EPNs possess vertical movement capabilities up to 10 cm in soil, which corresponds to the typical depth where these sedentary and cryptic insects can be found (Segura-León *et al.*, 2014).

Previous studies have evaluated EPNs against curculionids. For instance, in Táchira, Venezuela, three native *Steinernema* strains and two *Heterorhabditis* strains were tested against larvae of the pineapple weevil *Metamasius dimidiatipennis* (Coleoptera: Curculionidae). *Steinernema* sp.-075 was the most effective, achieving 95% larval mortality in 3.19 days at a dose of 500 IJs/larva. These findings support the idea that virulence and aggressiveness vary by strain (García-Caicedo *et al.*, 2013). This aligns with our results, suggesting that *H. bacteriophora* (C-23) may have adapted to short-cycle fly larvae and other soil organisms like ticks and white grubs, which are prevalent in its native habitat (Cruz-Vázquez *et al.*, 2003; Márquez, 2002). Although white grubs are not major pests, their presence is encouraged by high organic matter in the soil. García-Caicedo *et al.* (2013) argue that variations in EPN effectiveness are due to differences in pathogenicity and the susceptibility or resistance of

target insect populations. This could be linked to the ecological niche from which each isolate was obtained. For example, although *H. bacteriophora* (JAAN-19) was collected from a sugarcane field, it may never have interacted with *S. incurrens*, as this pest was primarily found in the regions of Las Paredes and Ayuquila, not near Autlán de Navarro, Jalisco where JAAN-19 was isolated (Grifaldo-Alcántara *et al.*, 2023).

During the evaluations conducted in this study, a clear relationship was observed between the number of nematodes applied and the increase in pest mortality, as well as the duration of their interaction. Among the isolates, *Heterorhabditis bacteriophora* (C-23) showed the greatest effect, achieving 50% mortality at a dose of 2,626 IJs/weevil with an LT_{50} of 17.8 days. In comparison, *H. bacteriophora* (JAAN-19) required 11,558 IJs/weevil with an LT_{50} of 28.2 days, and *H. bacteriophora* (AUME-20) needed 69,167 IJs/weevil with an LT_{50} of 26 days. In studies conducted in Colombia, the use of *H. bacteriophora* against the banana weevil *Metamasius hemipterus sericeus* (Coleoptera: Curculionidae) showed that a dose of 1,000 IJs/adult achieved an LT_{50} of 3.5 days and 80% mortality in 15 days (Jiménez *et al.*, 2012). The significant difference in LT_{50} between *H. bacteriophora* (C-23) (17.8 days) and the result for *M. hemipterus sericeus* (3.5 days) may be attributed to differences in insect size. While the banana weevil has a body length of <2 cm (Weissling *et al.*, 2003), the sugarcane weevil is smaller, measuring less than 1.3 cm.

Differences in infectivity may be attributed to host insect size, as suggested by Bastidas *et al.* (2014), and supported by Amador *et al.* (2015). In their evaluation of *Heterorhabditis atacamensis* CIA-NE07 against the banana weevil *Cosmopolites sordidus* (>1.5 cm in length), the main limiting factor was the nematodes' difficulty in penetrating the host, given that the mouthparts and spiracles were too narrow and the anus remained mostly closed. Jiménez *et al.* (2012) also noted that infective juveniles face challenges entering through natural openings and suggested that low mortality rates may result from limited host mobility. A similar behavior was observed during our evaluations of sugarcane weevils, which were active before exposure to EPNs. However, Ansari *et al.* (2006) argue that infection success is less dependent on host anatomy or mobility and more on the nematodes' ecological interaction and coexistence with the host. We partially agree with this interpretation, emphasizing both the host's defensive strategies (*e.g.*, limited openings and reduced movement) and the ecological compatibility between EPN strains and their target insect as key factors influencing infection outcomes.

Differences in infectivity, according to Bastidas *et al.* (2014), may be due to the size of the host insect. This is supported by Amador *et al.* (2015), who, in their evaluation of *Heterorhabditis atacamensis* CIA-NE07 against the banana weevil *Cosmopolites sordidus* (>1.5 cm in length), identified the primary barrier to infection as the nematodes' difficulty entering the host. The weevil's mouthparts and spiracles were too narrow, and the anus remained mostly closed. Similarly, Jiménez *et al.* (2012) reported that infective juveniles (IJs) struggle to enter through natural openings and suggested that low mortality may also result from the limited mobility of the host. This behavior was observed in our evaluations, as sugarcane weevils exhibited active movement before contact with EPNs. However, Ansari *et al.* (2006) argue that infection efficiency is not solely determined by host anatomy or activity, but by the ecological interaction and coexistence between EPN strains and their

hosts. We partially agree with this perspective, recognizing both the challenge nematodes face in penetrating a cryptic and low mobility host and the importance of host-pathogen ecological compatibility in determining infection success.

Although the evaluated isolates were native microorganisms from three different ecosystems, their interactions with hosts and development through multiple life cycles were also ecosystem specific. Therefore, as noted by Molyneux (1986), their thermal niche and life history with various hosts along with the physical and climatic conditions of each site likely influenced the success or failure of each isolate. Initially, it was hypothesized that *Heterorhabditis bacteriophora* (JAAN-19), isolated from sugarcane fields, would be more effective against *Sphenophorus incurrens*. However, *H. bacteriophora* (C-23), collected from cattle corrals, showed greater pathogenicity. This outcome suggests that C-23 may have interacted with hosts presenting more complex morphological, physiological, and behavioral barriers such as beetles from the white grub complex (*Phyllophaga*, *Anomala*, and *Cyclocephala* spp.), and coprophagous beetles (*Canthon indigaceus chevrolati*, *Digitonthophagus gazella*, and *Pseudocanthon perplexus*) involved in nutrient cycling and the incorporation of decomposing organic matter from vertebrate animals. This also includes ticks, for which the efficacy and persistence of EPNs in such environments have been studied (Alekseev *et al.*, 2006). These findings align with Gaugler and Kaya (1990), who emphasized that EPNs isolated from different geographic and climatic regions may behave differently as biological control agents.

Therefore, we propose that *Heterorhabditis bacteriophora* (C-23), which exhibits a "cruiser-forager" host-seeking strategy, could regulate sugarcane weevil populations under field conditions by locating the insect in its natural habitat. EPNs are known to possess chemoreceptors that guide them toward their hosts through volatiles such as CO_2, lactic acid, and insect feces (Kaya and Gaugler, 1993), enhancing their potential to effectively control different developmental stages of *Sphenophorus incurrens* within sugarcane crops. In a study by Delgado-Ochica and Sáenz-Aponte (2012), six EPN isolates were evaluated against the guava weevil, and *Heterorhabditis* sp. SL0708 was suggested for future field trials due to its effective host-searching behavior and high mortality against the pest. Similarly, we believe that the *H. bacteriophora* (C-23) isolate could be effective in controlling both larval and adult stages of *S. incurrens* under field conditions. Larvae may be especially susceptible due to their softer cuticle, limited movement, and more exposed natural openings such as the mouth, anus, and spiracles (Jiménez *et al.*, 2012). This is particularly relevant given that chemical applications have not effectively reduced weevil populations (Cerda *et al.*, 1999).

CONCLUSIONS

The insect demonstrated high resistance to EPNs, despite infective juveniles possessing an anterior tooth that aids in penetrating the host cuticle and overcoming its protective exoskeleton. Among the isolates, *Heterorhabditis bacteriophora* (C-23), collected from cattle farming areas, showed high virulence at a dose of 2,626 IJs/weevil. This effectiveness may be attributed to its interaction with other hosts (*e.g.*, coleopterans, dipteran pupae, ticks) with thick cuticles that coexist in the same bio ecosystem. However, we believe that the

virulence of all evaluated isolates could increase after several infective interactions (host life cycles), allowing the resulting progeny to become more adapted to the weevil host better understanding its immune system, evasion mechanisms, and behavior.

ACKNOWLEDGMENTS

We thank Ingenio Melchor Ocampo S.A. de C.V. in Autlán de Navarro, Jalisco, for their support, as well as engineers Vicente Abeldaño, Juan Carlos Anguiano Medina, and the head of the field department, Engineer Sinue Torres Hernández.

REFERENCES

Alekseev E., Glazer I. and Samish M. (2006). Effect of soil texture and moisture on the activity of entomopathogenic nematodes against female *Boophilus annulatus* ticks. *Biocontrol 51*(4): 507-518. https://doi.org/10.1007/s10526-005-2935-9

Amador M., Molina D., Guillen C., Parajeles E., Jiménez K. y Uribe L. (2015). Utilización del nematodo entomopatógeno *Heterorhabditis atacamensis* CIA-NE07 en el control del picudo del banano Cosmopolites sordidus en condiciones *in vitro*. *Agronomía Costarricense 39*(3): 47-60. https://doi.org/10.15517/rac.v39i3.21791

Ansari M.A., ALI F. and Moens M. (2006). Compared virulence of the Belgian isolate of *Steinernema glaseri* (Rhabditida: Steinernematidae) and the type population of *S. scarabaei* to white grub species (Coleopetera: Scarabaeidae). *Nematology* 30: 787-791. https://doi.org/10.1163/156854106778877938

Arnqvist G., Nilsson T. and Katvala M. (2004). Mating rate and fitness in bean weevils. *Behavioral Ecology* 16: 123-127. https://doi.org/10.1093/beheco/arh119

Arredondo-Bernal, H.C. y Rodríguez-del-Bosque, L.A. (2008). Casos de control biológico en México. Mundi Prensa México. México, D. F., México. 423 p.

Barbercheck, M. E. and Kaya, H. K. (1991). Effect of host condition and soil texture on host finding by the entomogenous nematodes *Heterorhabditis bacteriophora* (Rhabditida: Heterorhabditidae) and *Steinernema carpocapsae* (Rhabditida: Steinernematidae). *Environmental Entomology 20*(2): 582-589. https://doi.org/10.1093/ee/20.2.582

Barreto-Triana, N., Dos-Santos-Días, C. T. y Bento. S,J.M. (2014). Comportamiento reproductivo de la caña, *Sphenophorus levis* (Coleoptera: Curculionidae) en laboratorio. *Revista Colombiana de Entomología 40*(2): 265-271.

Cerda, H., Fernández, G., López, A., and Vargas, J. (1999). Olfactory attraction of the sugarcane weevil (Coleoptera: Curculionidae) to host plant odors, and its aggregation pheromone. *Florida Entomologist* 82: 103-112. https://doi.org/10.2307/3495841

CICOPLAFEST (Comisión Intersecretarial para el Control del Proceso y uso de Plaguicidas, Fertilizantes y Sustancias Tóxicas). 2022. https://www.dof.gob.mx/nota_detalle.php?codigo=5673267&fecha=05/12/2022#gsc.tab=0 Fecha de consulta 12 de mayo de 2024

CONADESUCA (Comité Nacional para el Desarrollo sustentable de la Caña de Azúcar). 2024. Ficha técnica del cultivo de la caña de azúcar (*Saccharum officinarum* L.). Gobierno de México,MEX. https://www.gob.mx/cms/uploads/attachment/file/141823/Ficha_T_cnica_Ca_a_de_Az_car.pdf Consultado el 15 de julio de 2023.

Cruz-Vázquez, C., Ramos, P.M., García-Vázquez, Z., Perales, S.C., Mata, D. (2003). Influencia de algunos factores climáticos en la distribución anual de la infestación por *Haematobia irritans* (Diptera: Muscidae) en un establo lechero de Aguascalientes, México. *Veterinaria México* 34: 315-322.

Delgado-Ochica, Y., y Sáenz-Aponte, A. (2012). Virulencia, producción y desplazamiento de nematodos entomopatógenos sobre larvas del picudo de la guayaba *Conotrachelus psidii* Marshall (Coleoptera: Curculionidae) en laboratorio. *Universitas Scientiarum 17*(3): 283-290.

Domínguez-Monge, S., Domínguez-Monge, M., Blanco-Méndez, T., Rosas-Porras, E., López-Vera, E., Campos-Figueroa, M. (2017). Ocurrencia de *Sphenophorus incurrens* Gyllenhal en la Región Centro de Veracruz, México. *Southwestern Entomologist 42*(3): 927-930. https://doi.org/10.3958/059.042.0334

Ferrer, F. y Salas, J. (2024). De los insecticidas al control biológico de plagas en caña de azúcar: una experiencia de medio siglo en Venezuela. *Ciencias Ambientales 58*(1) 1-16 https://doi.org/10.15359/rca.58-1.3

Girón-Pérez, K., Nakano, O., Silva, A.C. & Oda-Souza, M. (2009). Atração de adultos de *Sphenophorus levis* Vaurie (Coleoptera: Curculionidae) a fragmentos vegetais em diferentes estados de conservação. *Neotropical Entomology 38*(6): 842-846. https://doi.org/10.1590/S1519-566X2009000600019

Grifaldo-Alcántara, P.F., Beteran-Santana, C.H., Vargas-Madriz, H., Palomares-Pérez, M., Hernández-Contreras, S. and Azuara-Domínguez, A. (2023). Report of the Weevil *Sphenophorus incurrens* Gyllenhal in sugarcane cultivation at Jalsico, Mexico. *Southwestern Entomologist 48*(1): 279-282. https://doi.org/10.3958/059.048.0128

Jiménez, J.A., López, N.J.A. y Soto, G.A. (2012). Patogenicidad de dos nematodos entomopatógenos sobre *Metamasius hemipterus sericeus* (Coleoptera: Curculionidae). *Boletín Científico Centro de Museos Museo de Historia Natural 16*(2): 87-97. ISSN:0123-3068

Joyce, A.L., Sermeno-Chicas, M., Serrano-Cervantes, L., Paniagua, M., Scheffer, S.J. and Solis, M.A. (2016). Host-plant associated genetic divergence of two *Diatraea* spp. (Lepidoptera: Crambidae) stemborers on novel crop plants. *Ecology and Evolution 6*(23): 8632-8644. https://doi.org/10.1002/ece3.2541

Kaya, H.K. and Gaugler, R. (1993). Entomopathogenic nematodes. *Annual Review of Entomology 38*(1): 181-206. https://doi.org/10.1146/annurev.en.38.010193.001145

Koppenhöfer, A.M., Grewal, P.S. and Fuzy, E.M. (2006). Virulence of the entomopathogenic nematodes *Heterorhabditis bacteriophora*, *Heterorhabditis zealandica*, and *Steinernema scarabaei* against five white grub species (Coleoptera: Scarabaeidae) of economic importance in turfgrass in North America. *Biological Control* 38: 397-404. https://doi.org/10.1016/j.biocontrol.2005.12.013

Lewis, E.E. (2002). Behavioral ecology. In: Gaugler R (Ed.). Entomopathogenic Nematology. CABI Publishing. New York, 205-221. https://doi.org/10.1079/9780851995670.0205

Márquez, J.M. (2002). Metodología del muestreo de daño y pérdidas ocasionadas por rata en caña de azúcar. En: Memoria del XIV Congreso de Técnicos Azucareros de Centroamérica (ATACA) Guatemala, ATAGUA. pp. 68–74.

Pacheco, H.Ma. de L., Resendíz, M.F. y Arriola, P.V.J. (2019). Organismos entomopatógenos como control biológico en los sectores agropecuario y forestal de México: una revisión. *Revista Mexicana de Ciencias Forestales 10*(56): 1-29. https://doi.org/10.29298/rmcf.v10i56.496

Ramírez-Mora, E., Pérez-Vázquez, A., Landeros-Sánchez, C., Martínez-Dávila, J.P., Villanueva-Jiménez, A.A. y Lagunes-Espínoza, L. del C. (2018). Uso histórico de los plaguicidas en caña de azúcar del DR035 La Antigua, Veracruz. *Acta Universitaria* P.p. 42-49. https://doi.org/10.15174/au.2018.1644

Rodríguez-del-Bosque, L.A., Vejar-Cota, G., Vázquez-López, I., Villanueva-Jiménez, J.A., López-Collado, J., Alatorre-Rosas, R. y Hernández-Rosas, F. (2014). Plagas, pp. 147-186. In: Mata-Vázquez, H., Rodríguez-del-Bosque, L. A., Rodríguez-Morelos, V. H., y Vázquez-García, E. (eds), Manejo Integral de la Caña de Azúcar, SAGARPA-INIFAP-UANL.

Rodríguez, D., Torres, M., Uribe, L. y Flores, L. (2009). Susceptibilidad de los estadios L2 y L3 de *Phillophaga elenans* a una cepa nativa de *Heterorhabditis* sp. en condiciones de invernadero. *Agronomía Costarricense 33*(2): 171-182. https://doi.org/10.15517/rac.v33i2.6718

Rodríguez, D., Rodríguez, J., Rodríguez, D., Castillo, A., Enríquez, V., Milanés, N., Aguilar, N. y Herrera, A. (2011). Método en la determinación de presencia y daño de barrenadores, caso: Central Progreso, S.A. DE C.V. Veracruz, México. pp. 85. Tesis de Maestría, Universidad Veracruzana, México.

Sáenz-Aponte, A. y Olivares, W. (2008). Capacidad de búsqueda del nematodo entomopatógeno *Steinernema* sp. SNIO 198 (Rhabditida: Steinernematidae). *Revista Colombiana de Entomología 34*(1): 51-56. ISSN:2665-4385

Sánchez-Bolón, H. (2017). Desarrollo de un método para la detección y monitoreo del picudo de la caña en Tabasco. Tesis de Maestría, Colegio de Postgraduados, Campus Montecillo, Texcoco, Estado de México, 47 p.

Santiago-Zarate, I.M., Martínez-Damián, M.A., Cuevas-Alvarado, C.M., Valdivia-Alcalá, R., García-Hernández, M.I. y Hernández-Toscano, J. (2022). Productividad y cambio tecnológico en la agroindustria de la caña de azúcar en México. *Revista Mexicana de Ciencias Agrícolas 12*(6): 1005-1017. https://doi.org/10.29312/remexca.v12i6.2692

Segura-León, O.L., Hernández-Arenas, M., Cibrián-Tovar, J. and Romero-Nápoles, J. (2013). Damage diagnostic of *Sphenophorus incurrens* Gyllenhal (Coleoptera: Curculionidae: Dryophthprinae), in sugar cane in México. *Entomological Society of America Annual Meetings*.

Segura-León, O.L., Romero-Nápoles, J. y Díaz-Corro, L. (2014). El problema actual del picudo del tronco de la caña de azúcar, *Sphenophuros incurrens* Gyllenhal (Coleoptera: Dryopthoridae) en los estados de Morelos y Michoacán, México. Ponencia ATAM 2014.

San-Blas, E., Campos-Herrera, R., Dolinski, S., Monteiro, C., Andaló, C., Garrigos-Leite, L., Rodríguez, M.G., Morales-Montero, P., Sáenz-Aponte, A., Cedano, C., López-Nuñez, J.C., Del-Valle, E., Doucet, M., Lax, P., Navarro, P.D., Báez, F., Llumiquinga, P., Ruíz-Vega, J., Guerra-Moreno, A. and Stock, S.P. (2019). Entomopathogenic nematology in Latin America: A brief history, current research and future prospects, *Journal of Invertebrate Phatology* 165: 22-45, https://doi.org/10.1016/j.jip.2019.03.010

Stock, S.P. y Goodrich-Blair, H. (2012). Nematodos parásitos, patógenos y asociados de insectos e invertebrados de importancia económica. En: Lacey LA, editora. Manual de Técnicas en patología de Invertebrados. Prensa Elsevier; 375-425.

Sturhan, D. and Mracek, Z. (2000). Comparasion of the *Galleria* bating technique and a direct extraction method for recovering *Steinernema* (Nematoda: Rhabditida) infective-stage juveniles from soil. *Folia Parasitologica*, 47: 315-318.

Treverrow, N. L. and Bedding, R. A. (1993). Development of a system for the control of the banana weevil borer, Cosmopolites sordidus with entomopathogenic nematodes, pp. 41-47. In: R. Bedding, R. Akhurst and H.K. Kaya (eds.). Nematodes and the biological control of insect pests. Nematodes and the biological control of pest. Melbourne Australi.

Weissling, T., Giblin-Davis, R., Center, B., Heath, R., and Peña, J. (2003). Oviposition by *Metamasius hemipterus sericeus* (Coleoptera: Dryophthoridae: Rhynchophoridae). *Florida Entomologyst, 28*(2): 174-177. https://doi.org/10.1653/0015-4040(2003)086[0174:OBMHSC]2.0.CO;2

White, G. F. (1927). A method for obtaining infective nematode from cultures. *Science*, 66: 302-303. https://doi.org/10.1126/science.66.1709.302-a

Zelaya-Molina, L. X., Chávez-Díaz, I. F., Santos-Villalobos, S., Cruz-Cárdenas, C. I., Ruíz-Ramírez, S., Rojas-Anaya, E. (2022). Control biológico de plagas en la agricultura mexicana. *Revista Mexicana de Ciencias Agrícolas*, 27: 69-79. https://doi.org/10.29312/remexca.v13i27.3251