Colegio de Postgraduados

Registry of mermithid (Mermithidae) parasites of spittlebug nymphs (Hemiptera: Cercopidae) in sugarcane crops in Veracruz, Mexico

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ABSTRACT

Objective: To make known the presence of parasite mermithids of the spittlebug nymph in different sugarcane localities of the state of Veracruz, Mexico.

Design/methodology/approach: The nematodes were collected in sugarcane soils after observation of the emergence of the pest from the spittlebug nymphs (in situ), while some others were obtained after collecting spittlebug nymphs in the field and the pest's emergence in the laboratory. The death of the nematodes was provoked with hot water (sterile distilled, 60 °C), and they were measured and stored in Eppendorf tubes of 1.5 mL in formaldehyde 4% and kept in refrigeration at 4 °C.

Results: This report is presented as evidence of pest mermithids that emerge from the spittlebug nymph host in sugarcane crops in eight localities of the state of Veracruz. Although their identification at the species level could not be done, the length of each mermithid found was recorded, as well as its relationship with the host.

Limitations on study/implications: When the presence of mermithids was verified and their location was identified in diverse localities, the opportunity of continuing with the collection of specimens was justified and their identification could be formally performed, as well as the implementation of biological control studies with the use of these native nematodes; also, the exploration of their roles in the control of nymphs from the sugarcane crop.

Findings/conclusions: The presence of mermithid parasites of spittlebug nymphs in the sugarcane crop in eight localities in the mountainous region of Veracruz was verified *in situ*. Their emergence was classified initially on nymphs of the spittlebug (Hemiptera: Cercopidae) that belong to the genera *Aeneolamia* and *Prosapia*.

Keywords: natural control, *in situ*, nematodes, parasitic, spittlebug.

Citation: Hernández-Rosas, F., Pérez-Pacheco, R.2 Martínez-Martínez, R., Monteon-Ojeda, A., Hueso-Guerrero, E. J., Mendieta-Moctezuma, A., &Grifaldo-Alcántara, P. F. (2024). Registry of mermithid (Mermithidae) parasites of spittlebug nymphs (Hemiptera: Cercopidae) in sugarcane crops in Veracruz, Mexico. *Agro Productividad*. https://doi.org/10.32854/agrop.v17i11.2869

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

Received: March 27, 2024.
Accepted: October 16, 2024.
Published on-line: December XX, 2024.

Agro Productividad, 17(11). November. 2024. pp: 137-143.

INTRODUCTION

The spittlebug pest (Hemiptera: Cercopidae) acquired economic importance in the sugarcane crop (*Saccarum officinarum* L.) in Mexico at the start of the 20th century, and presently causes losses of up to 9 kg/ha^{-1} (De la Cruz-Llanas *et al.*, 2005). Their feeding begins from egg hatching until the passage of five instars,

where they remain in the root zone and suction the sap from the superficial roots and cause a "physiological disorder" (García *et al.*, 2007). As adults, they feed on the sap of leaves where they introduce their stylet and provoke lesions on the leaves known as "burns" (García *et al.*, 2007).

In Mexico, the presence of this pest involves climate triggers that favor its persistence within the crop, such as temperature (26-45 °C), clay soils, and the presence of natural hosts (grasses) (García-García *et al.*, 2006); as well as high precipitations (June-July) that mark egg hatching and give rise to the nymphs, also called "spittlebugs" (Enríquez *et al.*, 2002).

Control of this pest has required knowledge related to its population dynamics, diapause, chemical products, resistant varieties, and biological agents (Rodríguez *et al.*, 2003). In this strategy, the use of entomopathogenic nematodes has been considered as a viable alternative for their control within the sugarcane crop (Grifaldo-Alcántara *et al.*, 2019).

However, there are other types of nematodes, such as mermithids, which are long, thin and translucent worms that are highly specialized in parasitizing a broad range of invertebrates, and part of their biology involves pre-parasitic infectious juveniles, which enter the tegument until they reach the hemocele where they can finally kill their host (Platzer, 2007).

The findings of mermithids in spittlebug nymphs were reported since 1985 in Venezuela, where the presence of *Hexamermis dactylocercus* parasitizing nymphs of *Aeneolamia varia* (Cercopidae) was discovered, with a level of infection under natural conditions of 50% in nymphs collected in the field (Poinar Jr. and Linares, 1985). In Argentina, the presence of mermithids was reported which emerged from nymphs belonging to the genera, *Deois mourei* and *Notozulia entreriana* (Foieri, 2016). Meanwhile, in Mexico, Flores (1994) reported the first sightings of mermithids of the genus *Hexamermis* sp. as enemies of spittlebug nymphs in the sugarcane crop.

Therefore, the objective of this study is to make known the registry of mermithids that parasitize spittlebug nymphs, as well as the difference in size and their possible relationship with the host. The year and place of discovery in sugarcane crops for the state of Veracruz, Mexico, was also included.

MATERIALS AND METHODS

Records in this study are data accumulated since the year 2009 to the year 2022 in the sugarcane crop within different localities of the state of Veracruz, Mexico, to make known the presence of mermithids that parasitize the nymph (also called "spit") of the spittlebug. The detection and collection of organisms was through monitoring carried out by the field staff from each sugarcane mill within the crop for the control of the spittlebug pest. This was also done from direct collection of nymphs to perform the bioassays or identification of the pest. These nymphs were transported to the laboratory of Colegio de Postgraduados, Campus Córdoba, and fed with pieces of sugarcane plantlets to wait for the development and emergence of adults for their identification.

Both the collections of mermithids in the field and the organisms that emerged from the nymphs that were in the laboratory were placed in test tubes with sterile distilled water and

killed at 60 °C for two minutes; then the water was removed and they were placed on a flat surface and measured with a measuring tape to record the total length of the body (cm). To continue with their fixation and conservation, the mermithids were placed in Eppendorf tubes of 1.5 mL in formaldehyde 4% and kept refrigerated at 4 °C.

The mermithids that were recovered in 2019 when the nymphs emerged were kept in a plastic recipient (20×12×7 cm), to which water was added and a superficial layer of previously sterilized river stones, methodology suggested for the massive reproduction of mermithids that are mosquito parasites (Santamarina-Mijares and Berlini, 2000).

RESULTS AND DISCUSSION

In the state of Veracruz, since the year 2009 (Table 1), a registry was started of the presence of mermithids as natural parasites of spittlebug nymphs. The collection of these organisms was carried out with the help of entomological pliers.

Existing data about the mermithids detected are diverse (Table 1), among which there is information by the field staff from Ingenio CIASA (locality Comején), who observed the emergence of mermithids in "spittlebug" nymphs in situ. In the year 2013, in sugarcane areas of Ingenio Central El Potrero, after the collection of nymphs for their identification and then the development and emergence of adults in the laboratory, the exit of mermithids was detected in five nymph carcasses. The species *Aeneolamia albofasciata* was the one of largest population among the adults identified. After collecting spittlebug nymphs in the field to carry out bioassays of two entomopathogenic nematodes (*Steinernema* and *Heterorhabditis*) of this pest in the laboratory, Parada-Domínguez (2019) detected natural parasitizing in the nymphs evaluated of up to 10.42% (this is in five out of 48 nymphs).

The presence of mermithids was also recorded in municipalities such as Rancho Tablas, Estrella and Rincón del Otate (Table 1), where, after the evaluation of native entomopathogens (*Heterorhabditis indica* and *Steinernema* sp.) in two sugarcane mills (Grifaldo-Alcántara *et al*., 2019); the collection of spittlebug nymphs was carried out to identify adults and also to detect the natural emergence of mermithids in the laboratory, data not published in this report.

The last registry was in the year 2022, where after monitoring and placing green traps to capture the spittlebug in the field, the presence of two mermithids that emerged from nymphs was observed, with lengths of 18 to 21 cm, with these being the largest recorded in this study.

The study considers that the dissimilarity in the size of the mermithids found does not show a relation between the variety of sugarcane in the collection sites (Table 1), since the mermithids with lengths of 17 cm and 12 cm were related to the varieties, while the other mermithids, of 12, 14, 18, 21 cm, were present in CP varieties 72-2086; likewise, a relationship with the altitude (masl) is also not considered, or with the proximity between collection sites (Figure 1).

The presence of mermithids in the host *Aeneolamia varia* was recorded in Venezuela since the year 1985: the species *Hexamermis dactylocercus* whose length was 10 cm (Poinar Jr. and Linares, 1985). In contrast, the smaller measurements in this research were of 12 cm in the mermithids found in the municipalities Atoyaquillo, Estrella and Rincón

Table 1. Localities with presence of parasite mermithids of spittlebug nymphs in sugarcane crop and their relationship with spittlebug species (Hemiptera: Cercopidae) in Veracruz.

Localities	Municipality	Sugarcane mill	N	W	Altitude	Variety of sugarcane	Size of mermithids	Number of organisms	Year of presence	Individuals presents in spittlebug nymphs
1) Comejen	Hueyapan de Ocampo, Veracruz	CIASA	95° 11' 09"	18° 09' 38"	98 m	CP 72-2086	14 cm	2	Hernández-Rosas, communication personal, 2009	*Aeneolamia albofasciata* (before *A. postica*)
2) California	Amatlan de Reyes, Veracruz	Central El Potrero	18° 54' 11"	96° 51' 27"	586 m	ITV 290	17 cm	5	Hernández-Rosas, communication personal, 2013	*A. albofasciata* *Prosapia teapana* *P. simulans*
3) Atoyaquillo	Atoyac, Veracruz	Central El Potrero	18° 55' 38"	96° 51' 46"	553 m	Mex 69-290	12 cm	5	Parada- Domínguez *et al.*, 2014	*A. albofasciata*
4) Jareros	Ursulo Galvan, Veracruz	La Gloria	19° 23' 22"	96° 31' 04"	148 m	Mex 69-290	17 cm	3	Hernández-Rosas, communication personal, 2018	*A. albofasciata* *P. simulans*
5) Rancho Tablas	Acatlan de Pérez Figueroa, Oaxaca	Constancia	18° 33' 53"	96° 39' 00"	156 m	MEX 69290	17 cm	1	Grifaldo-Alcántara *et al.*, 2019	*A. albofasciata* *P. teapana*
6) Estrella	Acatlan de Pérez Figueroa, Oaxaca	Constancia	18° 33' 34"	96° 38' 00"	151 m	CP 72-2086 ITV 921424 MEX 69290	12 cm	3	Grifaldo-Alcántara *et al.*, 2019	*A. albofasciata*
7) Rincón del Otate	Amatlan de los Reyes, Veracruz	Central EL Potrero	18° 55' 25"	96° 53' 19"	557 m	MEX79-431	12 cm	2	Grifaldo-Alcántara *et al.*, 2019	*A. albofasciata* *A. contigua* *P. teapana* *P. simulans*
8) Paraíso la Reforma	Tezonapa, Veracruz	Central Motzorongo	18° 39' 28"	96° 44' 51"	296 m	CP 72-2086	18 cm y 21 cm	2	Hernández-Rosas, communication personal , 2021	*A. albofasciata* *P. simulans*

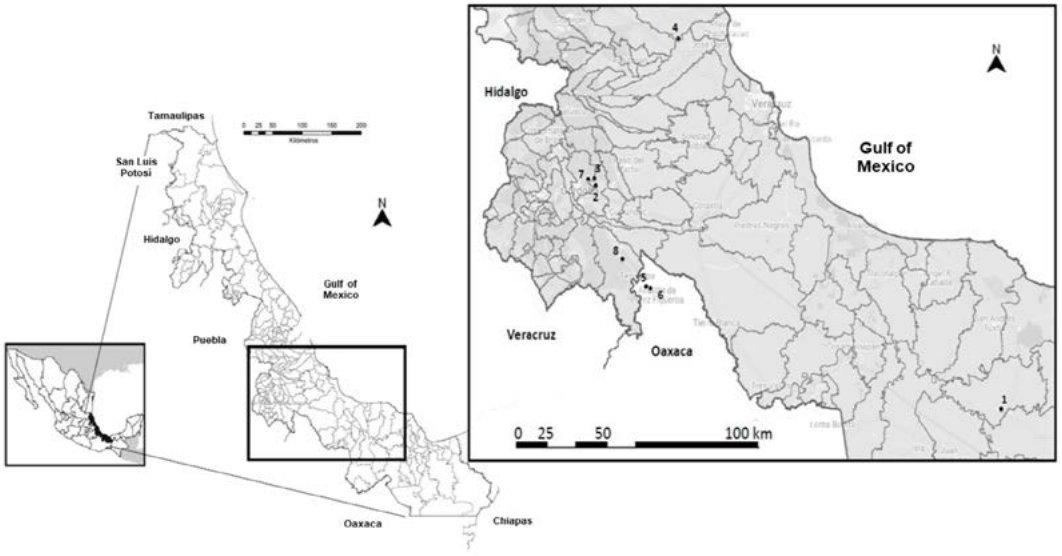

Figure 1. Presence of mermithids in the mountainous region of Veracruz, Mexico. Locality: 1) Comején, 2) California, 3) Atoyaquillo, 4) Jareros, 5) Rancho Tablas, 6) Estrella, 7) Rincón del Otate, and 8) Paraíso la Reforma.

del Otate Hueyapan de Ocampo, where the size of the two specimens was 14 cm; while the larger sizes were found in the municipality Paraíso la Reforma, 18 and 21 cm, followed by California, Jareros and Rancho Tablas with specimens that measured 17 cm. Regarding the interaction of mermithids and spittlebug species, the study assumes that it is possible for the presence of nematodes that range between 17 and 21 cm to be associated with populations of the genus *Prosapia*; meanwhile, the variation of mermithids between 12 and 14 cm would be more recurrent in populations of the genus *Aeneolamia*.

According to what was proposed by Welch (1965), these variations in the size of individuals could be linked to the size of the host that parasitizes it. Thus, it is considered possible for larger mermithids to be recovered from larger hosts/nymphs (*Prosapia*), while the small ones would be related to smaller hosts/nymphs (*Aeneolamia*); it is also regarded that they could be new species, for two reasons. First, because of the distance between *H. dactylocercus* discovered in Venezuela with a length of 10 cm, and the second, because of the variations in size (12 to 21 cm) that were observed in this study regarding the hosts where they were found.

Between the collections conducted in the years 2009 to 2021, all the nematodes discovered parasitizing spittlebug nymphs were killed with hot water and conserved in formaldehyde at 4%. However, the nematodes recovered in 2019 were kept in plastic recipients with sterile river stones and distilled water, trying to seek the survival of these specimens (Figure 2), following the methodology of parasite mermithids of mosquito (Santamarina-Mijares and Berlini, 2000), although the survival was not favorable. Therefore, this study agrees with Welch (1965), who mentioned that it is necessary to carry out studies related with the culture of mermithids, their propagation, and dissemination in the field.

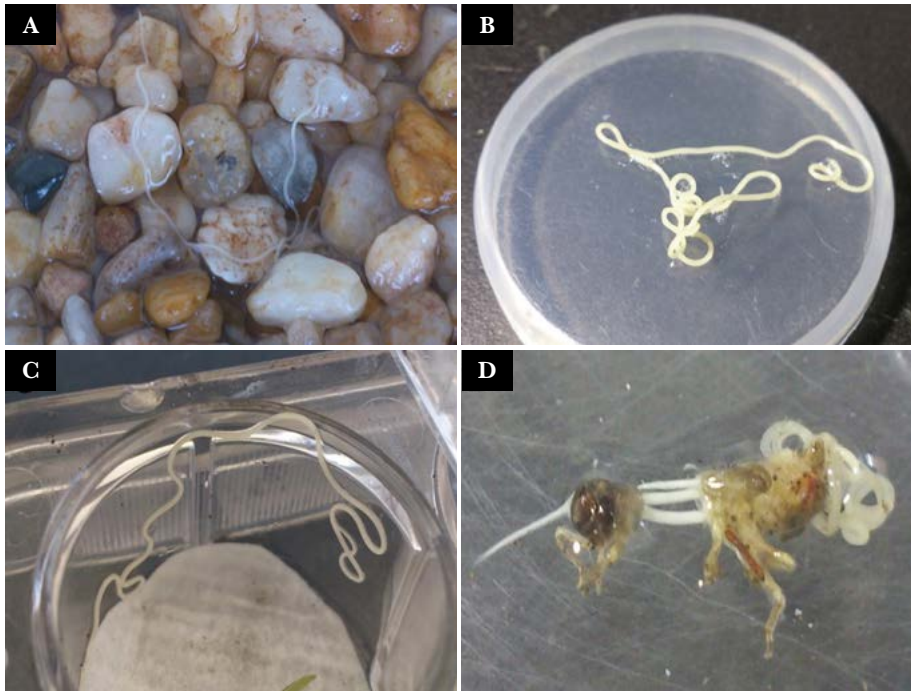

Figure 2. A) Evidence of mermithids, A) in river gravel; B and C) emerging from nymphs collected in the field; D) emergence in a spittlebug nymph (Hemiptera: Cercopidae).

Entomopathogenic nematodes present a favorable potential for control of spittlebugs (Grifaldo-Alcántara *et al.*, 2019); however, mermithids are used for biocontrol with high specificity, as is the case of mermithids for the control of mosquito larvae (Menéndez-Díaz *et al.*, 2018). Therefore, it is regarded that after the corrective identification of the mermithids present with molecular tools, a technique of multiplication, as well as their incorporation for the reduction of spittlebug nymph populations in sugarcane crops in Veracruz, could become an excellent alternative for the control of this pest.

CONCLUSIONS

The localities with sugarcane crops in the state of Veracruz are presented, where the presence of mermitids has been detected as parasitic nematodes of spittlebug nymphs. The study considers that there is no relationship between the variety of the sugarcane and the altitude (masl). However, hypothetically, it could exist between the length (cm) of the nematodes and the spittlebug genus parasitized. Regarding this, the mermithids of larger size could be related with the genus *Prosapia*, while for other smaller ones, the relationship would be with *Aeneolamia*.

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