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Original article

Natural enemies of *Steneotarsonemus spinki* (Acari: Tarsonemidae) in Campeche, Tabasco and Veracruz, Mexico, and evaluation of selected species for its biocontrol

Enemigos naturales de *Steneotarsonemus spinki* (Acari: Tarsonemidae) en Campeche, Tabasco y Veracruz, México y evaluación de especies selectas para su biocontrol

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ABSTRACT. Surveys were carried out in rice fields in the main areas where this crop is produced along the coast of the Gulf of Mexico to detect the *Steneotarsonemus spinki*

mite and its natural enemies, mainly predatory mites, and pathogenic fungi. In addition, the predatory capacity of one of the identified mites, *Gaeolaelaps aculeifer,* as well as the pathogenicity of the fungi *Hirsutella thompsonii*, *Isaria fumosorosea*, and *Metarhizium anisopliae,* against *S. spinki,* were evaluated in observation arenas. The presence of *S. spinki* was confirmed all along the Gulf of Mexico coast; the close species *Steneotarsonemus furcatus* was also identified infesting rice. The predatory mites *Lasioseius mcgregori*, *L. subterraneus*, *Gaeolaelaps aculeifer*, *Proctolaelaps curtipilis*, and *Neoseiulus paspalivorus* were identified. The *G. aculeifer* mite showed a limited predatory capacity, with daily consumption between four and 11 specimens; from the evaluated fungi, *H. thompsonii* showed the highest infectivity and potential to be used as biocontrol agent of *S. spinki,* with 46% infection of this mite.

Key words: Ascidae; Blattisosciidae; Deuteromycetes; Laelapidae; Melicharidae; *Oryza sativa*; Phytoseiidae; rice

RESUMEN. Se realizaron colectas en plantaciones de arroz en parcelas a lo largo de las principales áreas de producción en la costa del Golfo de México, para la detección del ácaro *Steneotarsonemus spinki* y de enemigos naturales, con énfasis en ácaros depredadores y hongos patógenos. Adicionalmente, se evaluó en arenas de observación la capacidad depredadora de una de las especies de ácaros identificados, *Gaeolaelaps aculeifer*, así como la patogenicidad de los hongos *Hirsutella thompsonii*, *Isaria fumosorosea* y *Metarhizium anisopliae,* sobre *S. spinki*. Se confirmó la presencia de *S. spinki* en los estados de Campeche, Tabasco y Veracruz; se identificó además a la especie cercana *Steneotarsonemus furcatus* infestando arroz. Se identificaron los ácaros depredadores *Lasioseius mcgregori, L. subterraneus, Gaeolaelaps aculeifer, Proctolaelaps curtipilis* y *Neoseiulus paspalivorus*. El ácaro *G. aculeifer* mostró una limitada capacidad depredadora, con consumo diario entre cuatro y 11 ejemplares; de los hongos probados, *H. thompsoni* mostró la mayor infectividad y potencial para usarse como agente de biocontrol de *S. spinki,* con 45% de infección de dicho ácaro.

Palabras clave: arroz; Ascidae; Blattisosciidae; Deuteromycetes; Laelapidae; Melicharidae; *Oryza sativa*; Phytoseiidae

INTRODUCTION

After wheat, rice (*Oryza* spp.) is the most used cereal in human nutrition worldwide. The best-known species of this crop is *Oryza sativa* L., 1753 (Degiovanni *et al*., 2010). Among the most important pests of rice is the *Steneotarsonemus spinki* Smiley, 1967 mite, which is commonly known as white mite or panicle rice mite. The reductions in yield attributed to this species have been estimated from 30 to 90% in China, and from 20 to 60% in Taiwan (Chen *et al*., 1980; Zhang *et al*., 1995). In the states of Campeche, Tabasco and Veracruz, Mexico, the surface of cultivated rice suffered a gradual reduction in the last ten years, down to 45%, attributed, at least in part, to the infestation of *S. spinki* (Salazar-Santiago, 2017).

This mite feeds by piercing the epidermal cells of the host plant, using its stylets to suck out the cell contents, resulting in the formation of brown spots with necrotic regions on the surface of leaf sheaths, panicles, and grains, as well as empty grains. It causes mechanical damage to the adaxial surface of the leaf sheaths, directly affects the rachis of the panicles, and influences the nutrient circulation mechanisms (Santos *et al*., 2004).

Although *S. spinki* was originally described with specimens collected in Louisiana, USA (Smiley, 1967), it appeared for the first time as an important pest in the Americas in Cuba in 1997 (Ramos & Rodríguez, 1998), and in the following years it spread throughout several Latin American countries (Navia *et al*., 2010). In Mexico, this mite was first detected in 2007 (Hummel *et al*., 2009) and has spread to wide areas along the coast of the Gulf of Mexico (Salazar-Santiago *et al*., 2019). Lack of proper management could cause this mite to spread to other rice production areas if it has not already spread to them.

In Cuba, this mite was observed to carry the spores of the *Sarocladium oryzae* (Sawada, 1976) fungus (Cabrera *et al*., 2005). As a result of infestation by *S. spinki*, infection by *S. oryzae*, or the combined attack by both species, the panicles present malformed, stained grains with a significant percentage of empty grains (Santos *et al*., 2004). However, Salazar-Santiago *et al*. (2019) did not find *S. oryzae* in rice fields in the state of Tabasco, Mexico, but they did observe symptoms such as those described above. They also did not find significant infections of other pathogenic fungal species in rice. With the previous results, these authors concluded that the emptying and staining of the grains are caused, at least in the study area, by *S. spinki* and not by its association with any pathogenic fungus.

Steneotarsonemus spinki is an extremely small mite; adult females measure approximately 274 x 108 µm, while adult males measure approximately 217 x 121 µm (Smiley, 1967), making them difficult to detect. Apart from the above, this mite hides deeply under the leaf sheaths and panicles, which makes its control difficult (Jaimez-Ruiz *et al*., 2015). Nevertheless, natural enemies have been detected that could be useful for its biological control, including mites from the families Ascidae, Laelapidae, and Phytoseiidae (Lo & Ho, 1984; Hummel *et al*., 2009; Quirós-McIntire & Rodríguez, 2010). Likewise, various species of entomopathogenic (more properly, acaropathogenic) fungi have been detected infecting *S. spinki* and creating important epizootics (Hummel *et al*., 2009). In rice fields on the coast of the Gulf of Mexico, Salazar-Santiago (2017) collected numerous isolates of *S. spinki* -pathogenic fungi. The species identified were all in the genus *Hirsutella*.

On the other hand, the Colegio de Postgraduados (Campus Campeche), in Mexico, has a collection of the generalist entomopathogenic fungi of the *Metarhizium anisopliae* (Metschnikoff, 1976) and *Isaria fumosorosea* (Wize, 1957). The *M. anisopliae* strain is widely used against rice pest insects under the trade name Ma005®; however, its effectiveness against *S. spinki* has not been evaluated.

The present work was developed with the objectives of identifying the predatory and pathogenic organisms of *S. spinki* in the most important rice-growing areas of the Coast of the Gulf of Mexico, which are in the states of Campeche, Tabasco, and Veracruz, and to evaluate the potential of select species of predators and pathogenic fungi to control *S. spinki*.

MATERIALS AND METHODS

Collection and identification of predatory mites associated with *S. spinki*. Collections were carried out in rice fields in the states of Campeche, Tabasco, and Veracruz, Mexico, during the autumn 2019 crop cycle. The data obtained in that crop cycle were complemented with collections made between 2014 and 2022. The study sites are shown in Table 1. The collected mites were from families that include species recognized as predators of other mites. The collections were always carried out where the mentioned mites were associated with *S. spinki* or at least that they were found in the same microhabitat. The mites were captured with a fine brush, using a stereoscopic microscope (10 to 40x).

Table 1. Collection locations in rice crops to obtain *Steneotarsonemus spinki* and its natural enemies.

State	Location	Coordinates
Campeche	Rancho Zináparo, municipality of Escárcega, Campeche	18.582283, -90.32927
	El Juncal, municipality of Palizada, Campeche	18.045194, -91.869611
Tabasco	Poblado C-26, in the land of Eliseo Jiménez, municipality of Huimanguillo, Tabasco	18.028269, -93.6534
	Poblado C-26, in the land of María Chablé, municipality of Huimanguillo, Tabasco	18.02641, -93.645534
Veracruz	Piedras Negras, municipality of Tlalixcoyan, lands of Chabelo Guzmán	18.762508, -96.24183 and 18.757034, -96.254715
	Piedras Negras, municipality of Tlalixcoyan, lands of Victorino Lozano	18.766024, -96.194565 18.76382, -96.18606 and 18.76301, -96.19422
	La Tranca, municipality of Tlalixcoyan, unknown owners	18.7656433, -96.251406 and 18.768362, -96.255196

The mites were preserved in 70% alcohol and mounted between slides and coverslips in Hoyer's liquid (Krantz & Walter, 2009) for subsequent observation in the phase contrast microscope and identification with the support of the relevant literature, appropriate for each cluster. The bibliographic sources used to identify each species are listed in Table 2. With additional specimens of each predatory species found, an attempt was made to found colonies to test their potential as natural enemies of *S. spinki*, for which the activities described below in detail were developed.

Table 2. Predatory mites associated with *Steneotarsonemus spinki* in rice in collections carried out in the states of Campeche, Tabasco, and Veracruz, Mexico.

Family	Scientific name	Collection location	Bibliographic source used for its identification
Blattisociidae	*Lasioseius youcefi* Athias-Henriot, 1959	Palizada, Campeche	Christian & Karg (2006)
	Lasioseius subterraneus Chant, 1963	Piedras Negras, Veracruz; Rancho Zináparo, Escárcega, Campeche	Christian & Karg (2006)
Laelapidae	*Gaeolaelaps aculeifer* (Canestrini, 1884)	Piedras Negras, Veracruz	De Moraes *et al*. (2022)
Melicharidae	*Proctolaelaps curtipilis* (Chant, 1958)	Piedras Negras, Veracruz	Chant (1963)
Phytoseiidae	*Neoseiulus paspalivorus* (De Leon, 1957)	Piedras Negras, Veracruz; Palizada, Campeche	De Leon (1957)

Collection and identification of pathogenic fungi of *S. spinki* in rice crops in the study area. During visits to rice fields aimed at collecting predators of *S. spinki*, mites of this species were carefully observed in search of specimens infected by fungi, which could look like mummies, with legs in an almost natural position and changes in the coloration, or mycelium that sprang from their bodies and spread radially. Mites suspected to be infected were collected along with a small

portion of the plant tissue on which they were perched, placed in Eppendorf tubes, and stored in a cooler for transport to the laboratory.

Once in the laboratory, a small portion of mycelium or the entire mite was taken and seeded in H medium, developed by McCoy *et al*. (1972) and modified by Cabrera *et al*. (2006), and the seeded specimens were incubated at 25°C, in the dark. They were checked daily to see if any opportunistic fungi were growing. When the growing fungi had the expected appearance for an acaropathogen, such as *Hirsutella* sp., they were reseeded in Petri dishes, 10 cm diameter, with H medium.

Pathogenic fungi of the genus *Hirsutella* were incorporated into the present study from a collection previously formed by M.A. Salazar-Santiago, collaborator in this study, with isolates taken from *S. spinki* in the state of Tabasco in 2015-2016. The identification of those isolates was carried out macroscopically and microscopically, as well as by amplification of the internal transcribed spacer of ribosomal DNA; they were preserved in 15% glycerin, at -70°C (Salazar-Santiago, 2017).

Assessment of predatory mites and acaropathogenic fungi for the biological control of *S. spinki*. A method was developed to establish colonies of each species of predatory mite, aimed at having abundant specimens to test their effectiveness for the biological control of *S. spinki*. To do this, colonies of *Tyrophagus* sp. mites were initially bred to use them as a food source for the predators, as follows. Approximately 500 g of moist, organic matter-rich soil was collected from under palm trees (*Phoenix canariensis* H. Wildpret, 1882) in Texcoco, Mexico (19.465390, -99.909071). A search for mites was carried out with a stereoscopic microscope, aimed at locating species of the Acaridae family. Some specimens of these mites were mounted between slides and coverslips with Hoyer's liquid (Krantz & Walter, 2009) and were identified as *Tyrophagus* sp. by coincidence with the genus diagnosis (Fan & Zhang, 2007).

To provide food for the *Tyrophagus* sp. mites, pet food kibbles (Ganador Pedigree Purina®, Mexico) were moistened and left in a plastic container with an airtight seal to encourage fungal growth. Incubation temperature was estimated between 22 and 26°C; inside the containers, HR was 100%.

Observation arenas were made with plastic containers with lids, 5 cm in diameter by 2 cm high, with three 1 cm diameter holes covered with mesh (25 µm opening) to allow air entry but prevent mites from escaping. Four kibbles were placed in each arena, two that were in their original presentation and two that were invaded by saprophytic fungi. Next, *Tyrophagus* sp. mites were transferred to the arenas using a fine brush. Each of these arenas was checked daily to see if the mites were still alive and if they were feeding. Likewise, two drops of water were placed on one of the kibbles to keep them moist, and they were kept at room temperature (between 22 and 26 °C). When the colonies began to grow, they were moved to a new container, which had the bottom completely covered with kibbles to allow the mite populations to continue to thrive. The mites were checked every third day and incubated at 25 ±1 °C and relative humidity of 50-55%. Kibbles were added to the arenas once a week to promote fungal growth.

To breed the predatory mites, hermetically sealed translucent plastic containers (8 cm in diameter, 5 cm in height) were prepared with a hole 2.5 cm in diameter in the lid, to which a mesh was glued to prevent the mites from escaping and allow the aeration. A plastic capsule (1.5 cm in diameter, 1 cm in height) with water-saturated cotton was placed inside each container to maintain high humidity and offer it as a drink for the mites. Then, kibbles that were highly infested with the mites that would be provided as food (*Tyrophagus* sp.) were placed in the containers. From rice plants infested by *S. spinki* and depending on their availability, between 10 and 13 mites,

preliminarily determined as predators, were transferred with a fine brush moistened with water into each of the containers, each mite was placed directly on the kibbles with abundant *Tyrophagus* mites. These containers were checked every 24 hours to see if these predators were still alive, in addition to moistening the cotton. They were regularly provided with kibbles infested with *Tyrophagus* sp. as food.

Acquisition of rice plants. Rice plants were collected from a field located in Piedras Negras, Veracruz (18.762508, -96.24183), which were in the initial stage of tillering, approximately 80 days old from sowing. These plants were transferred to pots with soil from the same plot and taken to an incubation chamber at a temperature of 25 ±2 °C with LED lighting and a 14:10 (light: dark) photoperiod. They were watered every third day and fertilized with Peters® (Tricel-20®, Cosmocel, S.A., Mexico) at a dose of 0.25g/L every week. These plants were infested with *S. spinki* specimens and were intended to obtain enough specimens of this species necessary to evaluate the impact of its natural enemies.

Predation tests in "sandwich" observation arenas. Arenas were made consisting of two 7.5 x 2.5 cm glass slides and an acrylic sheet of the same size with a central hole of 1 cm in diameter, a wet paper napkin, a layer of cotton, a portion of rice leaf sheath with high infestation by *S. spinki*, slightly larger than the hole in the acrylic sheet, and two paper clips. The sandwich arenas were assembled as illustrated by Overmeer (1985, in Fig. 2.1.4.1.5).

Once each arena or sandwich was assembled, the *S. spinki* specimens that had been confined in the hole were counted, separately by developmental stages. In the same hole, an adult female of the predatory mite was transferred with a fine brush, the second slide was quickly placed on the arena in preparation and pressed with the two clips, placed at both ends, to prevent the escape of the mites, both predator and prey. Ten arenas (replicates) were prepared with the only predatory mite that managed to establish a colony, *Gaeolaelaps aculeifer* (Canestrini, 1884); they were incubated at 25 °C in a plastic box with moist napkins to maintain relative humidity close to 100% and observed daily for 13 days. Ten other arenas were daily prepared to replace those under observation with new specimens of *S. spinki* as a food source.

To examine the arenas, each one was opened under the stereoscopic microscope, quickly to prevent the predator from escaping. With a wet brush, the mite was transferred to the new arena, in which the mites destined to be its prey had previously been counted. It was then immediately closed. Once the first arena was opened, its contents were observed: number of prey mites in each stage and whether there were eggs from the prey and the predator; the number of preyed mites was calculated as the difference between the number of mites of each stage, before and after the exposure to predators.

Characterization of *Hirsutella* sp. fungi. *Hirsutella* isolates that were stored in special tubes (cryovials) at -70°C and that had been obtained by M.A. Salazar-Santiago, collaborator of this study, were activated in Petri dishes with H medium. To do this, small portions of mycelium were sown under aseptic conditions in Petri dishes with H medium, as previously described. All the Petri dishes were incubated in a growth chamber at 25 °C to observe the mycelial growth of the colonies. Small portions of mycelium were taken from the aforementioned isolates, and microscopic preparations were made between slides and coverslips with lactic acid as mounting medium. They were confirmed to belong to the *Hirsutella* genus by observing the characteristic phialides (Minter & Brady, 1980).

The identification of the fungi was complemented molecularly by sequencing of the internal transcribed spacer gene (ITS). For this, total DNA was extracted using the method described by Freeman *et al*. (1993), and polymerase chain reaction (PCR) was performed to amplify

a segment of the ITS gene using the primers ITS1 and ITS4 (White *et al*., 1990). The PCR mixture was as follows: the dyNAzyme TM II (Thermo Fisher Scientific, Madrid, Spain) reaction mixture, 10 µL; molecular biology grade water, 4 µL; sense and antisense primers, 0.5 µL of each; and template DNA, 5 µL, for a reaction volume of 20 µL. The PCR was performed in a MyCycler™ (BIORAD Laboratories Inc., Hercules, CA, USA) thermocycler with the following program: 94 °C for 5 min; 30 cycles of 94 °C for 30 s, 56 °C for 30 s, and 72 °C for 1 min; 72 °C for 10 min; 4 °C indefinitely. The PCR products were visualized on a 1.5% agarose gel (IBI Scientific, Dubuque, Iowa, USA) in 1X TAE buffer plus 1 µL of ethidium bromide, subjected to electrophoresis at 90 volts for 30 min. The amplicons were sequenced by Macrogen Inc. The sequences were edited and assembled in the Mega 10.0.5 program and compared with sequences deposited in GenBank using the BLAST program. Multiple alignment was performed with the ClustalW program.

Pathogenicity test. The observation arenas called sandwiches were the same as those used to evaluate predation effectiveness. Entomopathogenic fungi of the species *M. anisopliae*, *I. fumosorosea*, and *Hirsutella thompsonii* Fisher, 1950, were applied to them; the first two were provided by J. Lara-Reyna; the third was isolate M-2 (B-1), selected from those obtained by M.A. Salazar-Santiago during a previous research work (Salazar Santiago, 2017), since it had the most profuse growth of all the isolates.

Suspensions of conidia of *M. anisopliae* and *I. fumosorosea* were prepared at a concentration of 10^6 conidia/mL in a 0.5% Tween® solution; the concentration was estimated using a Neubauer chamber. In the case of *H. thompsonii*, the culture obtained did not allow the same concentration to be reached, so it was applied at 10^3 conidia/mL. The application was done by spraying on a piece of rice leaf sheath infested with variable numbers of *S. spinki* specimens previously placed in the sandwich arena. An attempt was made to simulate a drip point application, and this arena was immediately closed with a slide, as previously described. The experiment was carried out with 10 replicates (sandwich arenas) per fungal isolate, including a control that was treated with a 0.5% Tween solution, in a completely randomized design.

The sandwich arenas were placed in a plastic container with moist napkins, incubated at 25 °C in the dark, and checked one and two weeks after fungi application. In each of these checks, an attempt was made to recover half of the *S. spinki* mites present and they were mounted between slides and coverslips with Hoyer's liquid to estimate the proportion of those that were infected by fungi, by observation under a phase contrast microscope, 400 and 1000x.

The sandwich arenas functioned as wet chambers, so it was expected that they would stimulate the development of pathogenic fungi and their infection in mites, but they surely also stimulated the development of saprophytic fungi already present in the leaf sheaths. A mite was determined as infected if the mycelium was growing profusely within its body, or diagnostic structures of the applied fungi, such as the phialides of *Hirsutella*, were clearly seen. Percent infestations among treatments were compared by the Kruskal Wallis test (P = 0.05), using the software SAS 9.4 (SAS Institute, 2010).

RESULTS AND DISCUSION

Identification of mites associated with rice, including those determined to be predators of *S. spinki*. The presence of *S. spinki* was confirmed in all sampled locations (Table 1), which confirms that this mite has spread widely in rice-growing areas after approximately 12 years of its establishment in Mexico. Additionally, the close species *Steneotarsonemus furcatus* de Leon, 1956, was identified in the same habitat in the town of La Tranca, Tlalixcoyan, Veracruz (18.7656433, -96.251406). *Steneotarsonemus furcatus* had already been reported as inhabiting rice in Cuba and

Brazil and apparently coexisting with *S. spinki* (Navia *et al*., 2006). The damage that *S. furcatus* causes to rice has not been determined. The list of predatory mites identified in this study, real or potential natural enemies of *S. spinki*, with their respective collection data, is shown in Table 2.

Four of the species mentioned in Table 2 have a history of their role in the biological control of pest mites. *Neoseiulus paspalivorus* (De Leon, 1957) has a wide global distribution, especially in tropical and subtropical areas (Demite *et al*., 2020) and has been tested in Brazil for the biological control of the *Aceria guerreronis* Keifer, 1965 mite, which causes coconut scab (Lawson-Balagbo *et al*., 2008a), but it has also been found in Cuba in rice, where its role in the control of *S. spinki* is unknown (Ramos & Rodríguez, 2004). *Gaeolaelaps aculeifer* (Canestrini, 1884) has a wide global distribution in temperate climates (Mahjoori *et al*., 2014) but has also been found in Colombia (Rueda-Ramírez *et al*., 2018). This mite is an efficient predator of insects in the soil that is produced commercially as a biological control agent (Gerson *et al*., 2003). *Lasioseius youcefi* Athias-Henriot, 1959 has a wide distribution in Africa, Asia, Europe, and North America (Negm, 2014). Lo and Ho (1984) observed that it preys on *S. spinki*, and Walter and Lindquist (1989) define this mite as omnivorous, feeding on both small animals and fungi. Finally, *Lasioseius subterraneus* Chant, 1963 was found in Baja California, Mexico (Chant, 1963), but it was also found in Brazil, inhabiting coconut palm (*Cocos nucifera* L., 1753) and associated with the mite *A. guerreronis* (Lawson-Balagbo *et al*., 2008a).

For its part, *P. curtipilis* was collected in sorghum imported from Mexico (Chant, 1958) and in Santa Cruz, California (Chant, 1963). There is no data about its diet or its potential as a natural enemy of any pest. The identification of *N. paspalivorus*, *G. aculeifer*, and *L. youcefi* are the first records in Mexico of the presence of these species.

Pathogenic fungi of *S. spinki* in rice crops in the study area, identified by traditional means and molecularly characterized. Five isolates of acaropathogenic fungi of *S. spinki* obtained by M.A. Salazar-Santiago during 2015-2016 were reactivated, no additional isolates were obtained in 2019. Their observation under a microscope confirmed them as *Hirsutella*. The Ma 034 and Isaria isolates of *M. anisopliae* and *I. fumosorosea*, respectively, massively cultured on wet rice grains, and provided by J. Lara-Reyna, were added.

The identification results by comparison with sequences deposited in GenBank (BLAST) are shown in Table 3, which allow us to confirm the identity of all the fungi that were part of the present study. In the case of *H. thompsonii*, these are close haplotypes and only isolates M-1 and M-3 are identical in the amplified sequence. Due to the presence of synnemata in isolate M-2 (B-1) grown in H medium, it is classified as the synnematous variety of *H. thompsoni*.

Table 3. Percentage of similarity of ITS gene segments amplified with the ITS1 and ITS4 primers, with segments deposited in GenBank. Only the highest similarity values are noted for each segment.

Isolate	Species identity in GenBank	% similitude	Access codes in GenBank
M-2 (B-1)	*Hirsutella thompsoni*	100	DQ345579.1
		100	KM652188.1
M-5	*Hirsutella thompsoni*	100	DQ345579.1
M-5 (B-1)	*Hirsutella thompsoni*	100	KM652186.1
M-3	*Hirsutella thompsoni*	100	KJ524673.1
M-1	*Hirsutella thompsoni*	100	KJ524673.1
Ma 034	*Metarhizium anisopliae*	100	MN592779.1
Isaria	*Isaria fumosorosea*	100	MN733178.1

Hirsutella nodulosa Petch, 1926, has been identified infecting *S. spinki* in Cuba (Cabrera *et al*., 2005), Costa Rica (León González & Avilés Chávez, 2011), and Sri Lanka (Cabrera *et al*., 2002), so the finding of *H. thompsonii* as the only species that infects *S. spinki* in the study area represents novel data and suggests that native strains acquired the ability to infect this mite, recently established in Mexico.

Predation test. A thriving colony of mites identified as *Tyrophagus* sp. was established. With these mites used as food for predators, only one colony could be established with abundant specimens of *G. aculeifer*. Daily observations of consumption, survival, and reproduction of this predator appear in Figs. 1 to 3. Fig. 1 shows that *G. aculeifer* maintained a daily consumption of between four and 11 specimens of *S. spinki*. Given the above, it can be said that the predator was able to feed on the prey that was offered to it. However, the specimens of this predator were dying throughout the days of confinement, so that after 13 days there was only one survivor per arena on average (Fig. 2). Most of the eggs were laid in the first days of confinement, suggesting that the females were already in a gravid condition before receiving *S. spinki* as food. The maximum number of eggs per female per day was two (Fig. 3), and six females from each replicate did not lay any eggs, so it is postulated that *S. spinki* is not a suitable diet for *G. aculeifer*. Alternatively, it is possible that the confinement conditions, especially the high humidity inside the sandwich arenas, were not favorable for the development of *G. aculeifer*. However, this species has been observed mainly in soil, where it is effective in controlling small arthropods (Gerson *et al*., 2003). Presumably, high relative humidity should not be a limiting factor for said mite.

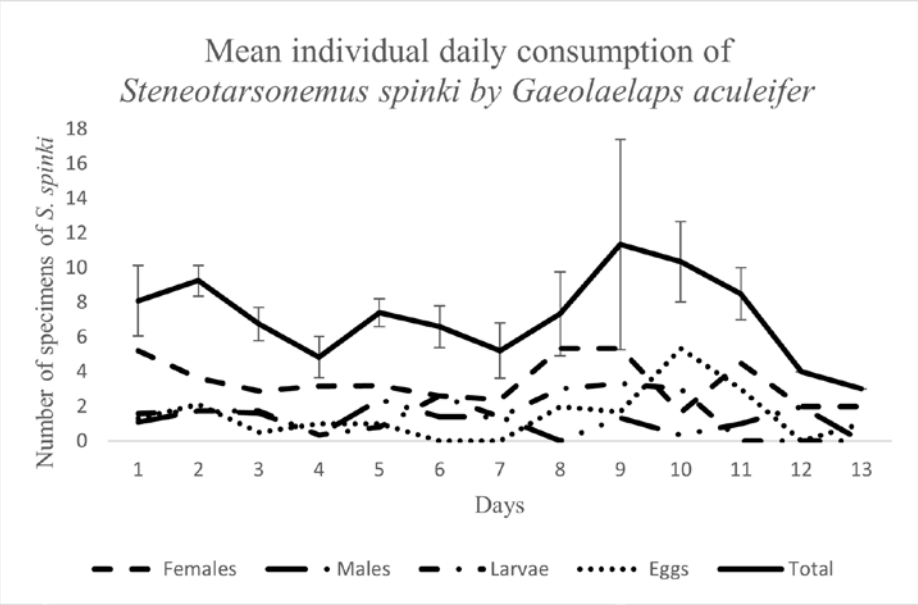

Figure 1. mean number of *Steneotarsonemus spinki* specimens consumed by adult females of *Gaeolaelaps aculeifer*, separated by stages, as well as total consumption, over 13 days in sandwich arenas. Vertical lines represent the standard error of each reading.

It was not possible to establish colonies of predatory mites of other species, which was particularly unfortunate for *N. paspalivorus* because it is a small and flattened mite, which would make it easier for it to enter the small spaces where *S. spinki* lives, as was observed when testing its effectiveness to prey *A. guerreronis* (Lawson-Balagbo *et al*., 2008b). According to McMurtry *et al*. (2013), *N. paspalivorus* belongs to subtype III-d in the lifestyle classification of phytoseiid mites. This group is characterized for being generalist predators that live in tight sites in monocotyledons,

precisely the microhabitat where it was found, so theoretically it should have the potential to control *S. spinki*. A closely related species, *Neoseiulus cucumeris* (Oudemans, 1930), is commercially raised using mites from the Acaridae family as food but prey on a wide variety of mites (McMurtry *et al*., 2015).

Similarly, and according to McMurtry *et al*. (2013), *L. youcefi*, *L. subterraneus*, *G. aculeifer*, and *P. curtipilis* are considered generalist predators, so it was expected that they would have the capacity to use *Tyrophagus* sp. as food.

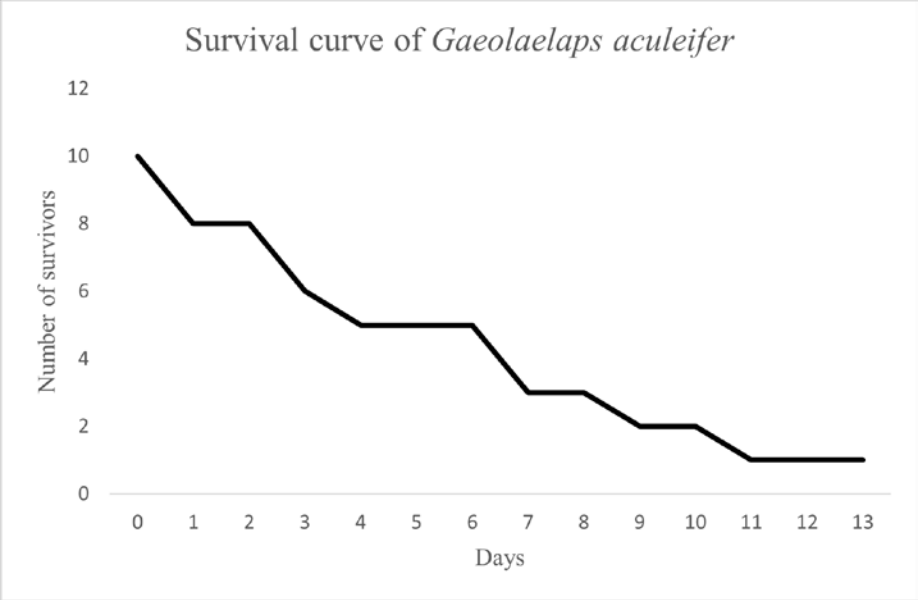

Figure 2. Survival curve of *Gaeolaelaps aculeifer* fed with *Steneotarsonemus spinki* in sandwich arenas.

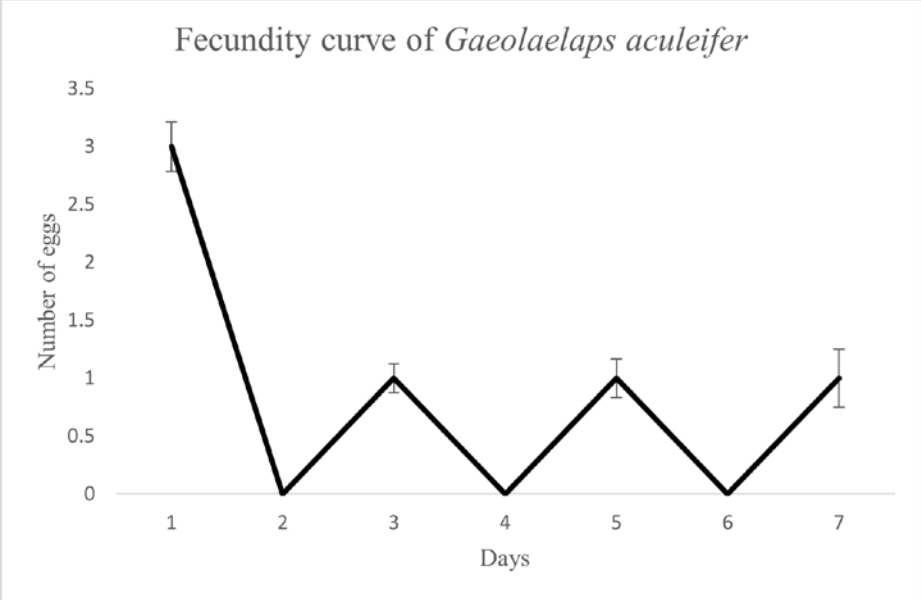

Figure 3. Average fecundity (number of eggs laid) over successive days of confinement of *Gaeolaelaps aculeifer* females in sandwich arenas, fed with *Steneotarsonemus spinki*. Vertical lines represent the standard error of each reading.

Fungal pathogenicity test. The percentage of mites infected by pathogenic fungi in the experiment in sandwich arenas is shown in Table 4. The so-called sandwiches are arenas in which the mites, the substrate, and the applied pathogens are confined in a narrow space, where the humidity is concentrated, so the conditions are theoretically favorable for the development of pathogenic fungi, but also for saprophytic fungi. Under these conditions, the three fungal species were able to infect *S. spinki* mites in variable proportions. A greater proportion of mites infected by fungi was observed in the first reading (seven days after application), which is tentatively attributed to the fact that as the days passed, the saprophytic fungi inhibited the action of the pathogens. It should be noted that *M. anisopliae* and *I. fumorosea* were applied at a concentration of 10^6 conidia/mL, while *H. thompsoni* was applied at a concentration 1000 times lower and, despite this, the latter fungus infected a higher percentage of mites, significantly different from the control one week after the inoculation. The first two species are considered pathogenic fungi for insects (Skinner *et al*., 2014), while *H. thompsoni*, like other species of the same genus, are considered specialists in mite infection (Wekesa *et al*., 2015). The isolate tested was obtained from *S. spinki*, which shows it to have greater potential to be used in the biological control of said mite. It is notable that during the observations carried out in 2019, no infections were observed in *S. spinki* caused by *Hirsutella* spp., contrary to the observations of Salazar-Santiago (2017), who obtained several isolates of species of this genus in natural infection. This suggests that infections, and possibly epizootics, may occur erratically and may not be a natural control mechanism for *S. spinki*. However, this does not exclude that activities can be carried out to promote the action of said fungus, such as its mass production and application in the field. The data from Cabrera *et al*. (2005), who associate rice emptying with the *S. oryzae* fungus in Cuba, would motivate producers to use fungicides to control this problem. Apparently, the situation in Mexico is different, since Salazar-Santiago *et al*. (2019) showed that emptying is caused by the *S. spinki* mite alone, so stopping the application of fungicides can be a resource to facilitate the development of epizootics caused by *Hirsutella* spp.

Table 4. Percentage of mites identified as infected by pathogenic fungi in sandwich arena tests.

Species	**Week 1**	**Week 2**
Hirsutella thompsoni	46	10
Metarhizium anisopliae	43	10
Isaria fumosorosea	20	5
Control	0	0

In summary and by way of conclusion, *H. thompsonii*, or some species of this genus, among those identified, is the one that shows the greatest potential to be tested in the biological control of the panicle rice mite. It is not an easy species to cultivate (Wekesa *et al*., 2015), so research on its mass production and application methods is a challenge to face. For its part, it is suggested to continue research on the predatory capacity of *N. paspalivorus*, due to its wide distribution and close association with *S. spinki*.

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