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In vitro predatory activity of *Lasioseius penicilliger* (Arachnida: Mesostigmata) against three nematode species: *Teladorsagia circumcincta*, *Meloidogyne* sp. and *Caenorhabditis elegans*

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Abstract

The aim of this study was to evaluate the predatory behavior *in vitro* of the mite *Lasioseius penicilliger* on 3 nematode species: *Teladorsagia circumcincta* (L_3) (a sheep-parasitic nematode), *Meloidogyne* sp. (J_2) (a plant-parasitic nematode), and on various developmental stages of *Caenorhabditis elegans* (a free-living nematode). The coincubation of mites and nematodes was individually assessed in 2% water agar placed in plastic Petri dishes (2 cm x 1 cm diameter). One thousand nematodes of a species and 5 mites were placed into each plate (10 replicates) and incubated for 5 days at room temperature (18-25ºC). *L. penicilliger* showed predatory behavior against the 3 assessed nematode species. The percentages of predatory activity recorded were 95.1, 80.5 and 79.3 against *Meloidogyne* sp., *C. elegans*, and *T. circumcincta*, respectively ($P \leq 0.05$). These results suggest that *L. penicilliger* has important potential as a biological control agent of parasitic nematodes.

Keywords: Predatory mite; Parasitic nematodes; Neotropical

Introduction

The parasitic nematodes are responsible for severe diseases to plants and animals and are a major concern to the livestock and agricultural industries (Fitzpatrick, 2013). The nematode *Teladorsagia circumcincta*, for example, is one of the most economically important parasitic nematodes of sheep in cool temperate regions (Gossner *et al.*, 2012). For this reason, farmers use different chemicals for controlling nematode parasites, although the indiscriminate use of drugs against parasitic nematodes and the ensuing development and growth of drug resistance in the parasites have led researchers worldwide to search for new control strategies (Kaplan, 2004; Davies and Spiegel, 2011; Good *et al.*, 2012; Torres-Acosta *et al.*, 2012b).

Therefore, the need has arisen for alternatives to chemical control such as biological control, which is a relevant option (Sayre and Walter, 1991; Timper, 2011). With respect to the economic importance of parasitic nematodes of ruminants, the need for molecular tools to specifically diagnose nematode infections for refined investigations of parasite epidemiology and drug resistance detection in combination with conventional methods must be emphasized (Roeber *et al.*, 2013).

Additionally, the production of different crops is affected by a variety of pathogenic organisms that seriously affect the production, development and plant vigor (Back *et al.*, 2002). Among these agents are the phytonematodes, which cause diverse symptomatology depending on the genus and species of nematodes, and that may affect different parts of the plant. The degree of pathogenicity depends on the aggressiveness of the strain as well as the anatomical and physiological adaptations of each plant to parasitism (Dutta *et al.*, 2011). In this context, *Meloidogyne* spp., which belongs to the group of gall-forming nematodes, is considered one of the most important pests in various crops, primarily in tropical and subtropical countries where it is widely distributed (Luc *et al.*, 2005), causing severe annual economic losses estimated at $125 million globally (Hodda and Cook, 2009; Safdar and McKenry, 2012; Sikora and Fernandez, 2005). As in the case of ruminant parasitic nematodes, alternative control methods, such as biological control approaches, are urgently needed to alleviate the huge economic burden that these parasitic nematodes cause to the farming industry (Van der Putten *et al*., 2006).

Nematodes in the soil have several natural enemies, such as viruses, protozoa (Bjornlund and Ronn, 2008), flatworms, insects, tardigrades (Sayre and Walter, 1991), nematode "predators" of other nematodes (Bilgrami, 2008), bacteria, nematophagous fungi (Mendoza de Gives and Torres-Acosta, 2012) and mites (Aguilar-Marcelino *et al.*, 2014).

Mesostigmata mites of the genus *Lasioseius* (Berlese, 1916) are distributed worldwide and belong to the Family Ascidae (Berlese). The species of this genus are considered predators and can be found on a variety of substrates, such as soil, litter and in association with insects and vertebrates (Walter and Lindquist, 1997).

In particular, the species *L. penicilliger* has advantages as a potential biological control agent due to characteristics such as its short life cycle, and parthenogenetic reproduction, which allows for medium-term population increases. It is important to note that the *L. penicilliger* used in the present study has been maintained in the laboratory using nematodes as food for 5 years. This may be important for selecting mites with preferences for parasitic nematodes as food. There have been few studies on the use of mites as control agents of parasitic nematodes. The aim of the present investigation, therefore, was to evaluate the *in vitro* predatory activity of *L. penicilliger* on *T. circumcincta* (L_3), *Meloidogyne* sp. (J_2) and *C. elegans* to test the hypothesis that this mite feeds differently on nematodes depending on their size and on the presence or absence of an outer cuticle.

Material and methods

The origin of biological material used was as follows:

Mite

Lasioseius penicilliger (Arachnida: Mesostigmata) was isolated from soil samples in Morelos, Mexico, in 2009 and identified according to Hughes (1976). Since then, the mite strain has been kept in the laboratory of Helminthology of CENID-PAVET by culturing in Petri dishes (2 cm diameter and 1 cm high) containing 2% water agar at room temperature, (27 ± 2ºC) under dark conditions (Bilgrami, 1994).

Panagrellus redivivus (Nematoda) were used as food, once a week, for the mites in the culture dishes. Adult mites, both males and females, were randomly used.

Nematodes

- *Teladorsagia circumcincta*. This nematode species was collected from a naturally parasitized deer at Guerrero state, Mexico, in 2011 (Liébano-Hernández, unpublished) and was identified according to its morphological characteristics (Indre *et al.*, 2011; Van Wyk *et al.*, 2013). It has since been maintained, as a pure isolate, at the CENID-PAVET by continuous passages into susceptible young sheep.
- *Meloidogyne* spp. This nematode was isolated from infected tomato plants (*Lycopersicon esculentum* Mill) from Jojutla Municipality, Morelos state, Mexico. This strain was cultured under greenhouse conditions at Jiutepec, Morelos state, Mexico, and was maintained by successive passages in tomato plants under controlled conditions.
- *Caenorhabditis elegans.* The strain N2, variety Bristol, was used; this nematode was cultured in Petri dishes containing NGM (nematode growth medium). As a first step, a wild *Escherichia coli* commercial strain (ER2738, New England The Biolabs) was grown in NGM for 2 h at 37ºC. An abundant nematode population was achieved by transferring them to new Petri dishes containing bacteria growing on NGM for 3 days (Carvalho *et al.*, 2014).

Experimental design

The predatory capacity of mites against nematodes was assessed using plastic Petri dishes (2 cm diameter x 1 cm high). Each Petri dish was considered as one experimental unit, which contained 2% bacteriologic agar in water (WAPD). During the experiment the dishes were kept at room temperature (18-25ºC).

The experiment consisted of placing 5 *L. penicilliger* adult and 1000 nematode larvae in each WAPD and 10 replicates of each treatment were made. The treatments were established as follows: S1: *T. circumcincta* (L3); S2: *Meloidogyne* sp. (J2); S3: *C. elegans*. Each WAPD was incubated for 5 days. At the end of this period, the mites were manually separated from every WAPD and the WAPD was washed with running water to collect the larvae. After 3 washings, nematodes of each series were recovered by the Baermann funnel technique after 12 h (Thienpont *et al.*, 1986).

Nematodes were then counted by placing ten 5 µL aliquots on a glass slide and examining them under an optical microscope (10X). After data recording, survival rate and the predation percentage of *L. penicilliger* on each nematode species was estimated as follows:

$$\text{Survival rate} = \text{number of recovered larvae} / 1000$$

$$\text{Percentage of predation} = \frac{\text{mnn in control group} - \text{mnn in treated group}}{\text{mnn in control group}} \times 100$$

Where mnn = mean of the number of nematodes

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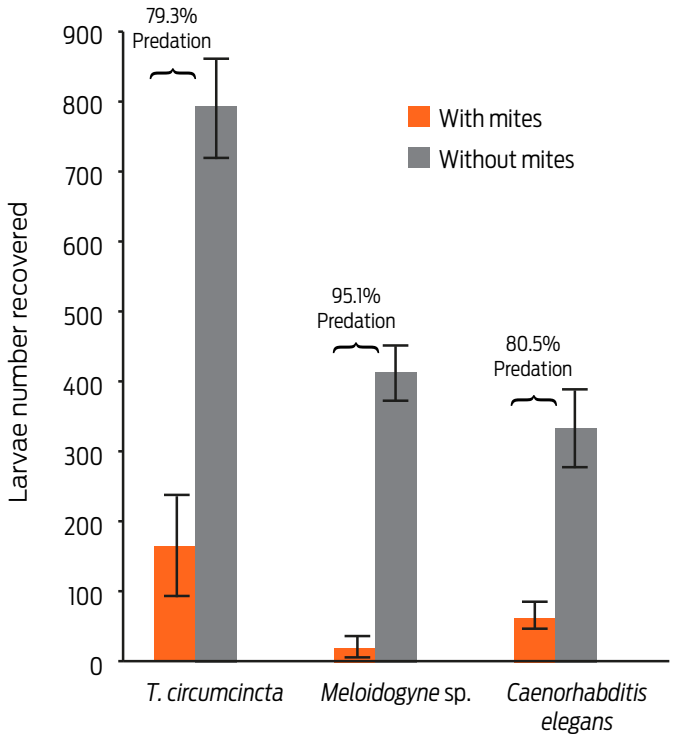

Figure 1. Recovered larvae (out of 1000) and mean percentages of predation of *L. penicilliger* on *Teladorsagia circumcinta* (L_3), *Meloidogyne* sp. (J_2), and *Caenorhabditis elegans* (different developmental stages) after 5 days of coincubation *in vitro*. Each point represents the mean ± standard deviation (n = 10).

Percentage of predation = [(average number of nematodes without mites - average number of nematodes co-incubated with mites) / average number of nematodes without mites] x 100

Statistical analysis

The data on survival rate were normalized using the arc-sine square root transformation and analyzed as a completely randomized design in a factorial arrangement of treatments (3 nematode species x 2 levels of mites – absence/presence). Means were compared using the Tukey test (SAS, 1998). A $P \leq 0.05$ value was considered as significant.

Results

After 5 days of nematode-mite coincubation, the numbers of recovered larvae (mean ± standard deviation) in control (C) and treated (T) groups were 792 ± 233 (C) and 164 ± 262.9 (T) for *T. circumcincta*; 415 ± 117.9 (C) and 20 ± 34 (T) for *Meloidogyne* sp., and 335 ± 166.7 (C) and 65 ± 66 (T) for *C. elegans*. The predation percentage of *L. penicilliger* was 79.3% on *T. circumcincta*, 95.1% on *Meloidogyne* sp., and 80.5% on *C. elegans* (Fig. 1). Notably, none of the mites died during the experiment (data not shown).

Table 1 shows the results from the analysis of variance. As expected, survival rate was always lowest in the presence of mites ($P < 0.05$). In the absence of mites, *T. circumcincta* had a higher survival rate than the other 2 nematodes, possibly because *Meloidogyne* sp. and *C. elegans* were completing their cycles and had no food during the 5 days of testing, whereas *T. circumcincta* was in its infective stage and did not need food. In the presence of *L. penicilliger*, the survival rate of *C. elegans* was similar to that of *Meloidogyne* sp. and *T. circumcincta* ($P > 0.05$), but the survival rate of *Meloidogyne* sp. was significantly lower than that of *T. circumcincta* ($P < 0.05$).

Discussion

Mites are being considered as promising bio-control agents of a number of agriculture pests (Chen *et al.*, 2013). The results of this study support this strategy on the basis that *L. penicilliger* was able to prey on the 3 different assessed nematodes, regardless of their taxonomic origin.

The survival rate of *Meloidogyne* sp. (J_2), however, was lower than that of *T. circumcincta* (L_3), suggesting a selective predatory behavior of mites against different nematode taxons that may be related to differences between the surface structure of plant parasitic nematodes (*Meloidogyne* sp.) and animal parasitic nematodes (*T. circumcincta*) (Gravato-Nobre and Evans, 1998). Raleigh *et al* (1996), for example, found the presence of a sheath in ruminant parasitic nematode larvae, which acts as a protective coat. Alternately, other members of genus *Lasioseius* spp.,

Table 1. Mean survival rate of *Teladorsagia circumcinta* (L_3), *Meloidogyne* sp. (J_2), and *Caenorhabditis elegans* (different developmental stages) after 5 days of *in vitro* coincubation with *L. penicilliger*.

Nematode species	Mite level	n	Survival rate[1] Mean ± SD
T. circumcinta	Absence	10	0.79 ± 0.22^{a}
	Presence	10	0.16 ± 0.26^{c}
Meloidogyne sp.	Absence	10	0.42 ± 0.12^{b}
	Presence	10	0.02 ± 0.03^{d}
C. elegans	Absence	10	0.34 ± 0.17^{b}
	Presence	10	0.07 ± 0.07^{cd}

[1]Survival rate = number of recovered larvae / 1000

e.g., *L. subterraneus*, have shown an enormous voracious activity against root-knot nematodes (Walter *et al.*, 1993). However, there is thus far very limited information about the feeding habits of *L. penicilliger* as a predatory mite of animal parasitic nematodes.

This species has recently displayed a lethal potential *in vitro* activity against *H. contortus* infective larvae (Aguilar-Marcelino *et al.*, 2014). Some information about the predatory activity of other *Lasioseius* species has been recorded against economically important plant-parasitic nematodes. For instance, *L. scapulatus* showed 99% *in vitro* predatory activity against *Aphelenchus avenae* (Imbriani and Mankau, 1983). With respect to the predatory activity of *L. penicilliger* against animal parasitic nematodes, a recent *in vitro* study showed 80% predation of this mite against *Haemonchus contortus* infective larvae (Aguilar-Marcelino *et al.*, 2014). Such observations suggest that perhaps *L. penicilliger* acts similarly against other members of the Trichostongylidae family. These results also indicate a high predatory activity of *L. penicilliger* on the 3 assessed nematode species.

The fact that 2 species of nematodes assessed in the present study, *T. circumcincta* and *Meloidogyne* sp., are important pathogens for ruminants and plants may have important implications on further studies searching for a possible application of biologic control agents against both animal and plant nematode plagues.

In this regard, it is important to emphasize that the natural habitat of infective larvae of *T. circumcincta* and the other members of the group of ruminant parasitic nematodes is within fecal matter. An increase in predacious mite populations has been achieved with organic manure, in studies in which predacious mites were used for the control of citrus nematodes, (El-Banhawy *et al.*, 1997). Therefore, perhaps one possible application of predacious mites, *ie. L. penicilliger*, for controlling ruminant parasitic nematodes may be through their use under field conditions on feces. This is, however, currently only speculation because the results were obtained *in vitro* and should be taken with caution. On the other hand, the fact that *L. penicilliger* acted against *C. elegans* (a free-living nematode) could be an undesirable feature. Much work is thus needed to further establish the potential of mites as biocontrol agents, considering that *L. penicilliger* is able to feed on both animal and plant parasitic nematodes.

Conclusions

The present research revealed important *in vitro* predatory activity by the mite *L. penicilliger* against *T. circumcincta* infective larvae. At this time, it is unclear how biological control using mites could reduce the parasitic larvae population in the field.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

Author contributions

Noemí García Ortiz and María Eugenia López Arellano: Conducted the experiment and critically reviewed and approved the manuscript for publication.

Liliana Aguilar Marcelino: Designed the experiment and critically reviewed and approved the manuscript for publication.

Pedro Mendoza de Gives: Analyzed the data and critically reviewed and approved the manuscript for publication.

Carlos Ramón Bautista Garfias: Analyzed the data and drafted and approved the manuscript for publication.

Roberto González Garduño: Reviewed the statistical analysis and drafted and approved the manuscript for publication.

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