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Sancassania mycophaga (Acari: Acaridae) as a regulatory agent for *Nacobbus aberrans* (Tylenchida: Pratylenchidae)

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ABSTRACT

Background/Objective. Nematophagous mites represent a potentially effective tool in integrated management programs for plant-parasitic nematodes. The objective of this study was to evaluate the predatory capacity of the nematophagous mite *Sancassania mycophaga* against the plant-parasitic nematode *Nacobbus aberrans* under laboratory conditions.

Experimental development. Two independent experiments were conducted. For this purpose, the experimental unit consisted of a pot with a pepper plant (*Capsicum annuum*), in which 500 or 1000 J2 juveniles of *N. aberrans* were inoculated. Four treatments were evaluated: (1) control (nematodes only), (2) simultaneous inoculation of mites and nematodes, (3) inoculation of nematodes five days after mites, and (4) inoculation of nematodes 10 days after mites. In all treatments, five mites were introduced per pot. The variables were: percentage of J2 penetration in roots, number of galls, eggs, and abundance of mites. The data were subjected to ANOVA and comparison of means (LSD, $p < 0.05$).

Results. Twenty-one days after the establishment of the trials, a significant reduction in nematode penetration was observed in all treatments with mites, compared to the control. In the experiment with 500 J2, the percentages of root penetration were 1.0, 3.4, and 2.5% for treatments 2, 3, and 4, respectively. In the experiment with 1000 J2, the penetration percentage was 21.3 and 23.8% for treatments 2 and 3. From day 14 onwards, the mite population showed sustained growth, increasing from an initial five individuals to more than 25 at 21 days (>400%) and more than 35 at 45 days (>600%). No significant differences in the number of galls or eggs were detected at 45 days.

Conclusions. *S. mycophaga* reduced the penetration of *N. aberrans* into pepper roots. With 500 J2, the relative reduction ranged from 42 to 83%, while with 1 000 J2 it varied between 42 and 49% compared to the control. In addition, the mite managed to establish itself and multiply in the experimental system. These results support its potential as a biocontrol agent for *N. aberrans*.

Keywords: Mite, Predation, Nematode, Phytoparasite, Biocontrol

INTRODUCTION

The nematode known as the "false root-knot nematode", *Nacobbus aberrans*, is considered a pest of economic importance due to the significant damage it causes and its wide range of hosts, which includes chili (*Capsicum annuum*), tomato (*Solanum lycopersicum*), potato (*Solanum tuberosum*) and bean (*Phaseolus vulgaris*) (Manzanilla-López *et al*., 2002; Manzanilla-López, 2010).

The infested plants develop galls throughout the root system, the shape and size of which vary over the course of the cropping cycle (Del Prado-Vera *et al*., 2018). In initial stages, the galls are small, slightly swollen and nodular in appearance, and are located mainly on secondary roots (Manzanilla-López *et al.*, 2002). As the nematode develops and the infestation increases, the galls become larger, deformed and woody, affecting wide areas of the root system (Marro *et al*., 2018). These structures interfere with the absorption of water and nutrients, compromising the functioning of the vascular system and significantly reducing the growth and productivity of the plant (Nicol *et al*., 2011; Radwan *et al.*, 2012).

Diverse studies have documented the potential of certain predatory mites on the biological control of phytoparasitic nematodes (Stirling, 2014; Abou El-Atta and Osman, 2016; Stirling, 2017; Aguilar-Marcelino *et al*., 2020). For example, *Lasioseius penicilliger* (Mesostigmata: Blattisociidae) displayed an ability to attack different parasitic nematodes, including *Teladorsagia circumcincta* (Strongylida: Trichostrongylidae), *Caenorhabditis elegans* (Rhabditida: Rhabditidae) and species of the *Meloidogyne* genus (Tylenchida: Heteroderidae) (García-Ortiz *et al*., 2015). Likewise, *Sancassania berlesei* (Astigmata: Acaridae) displayed efficient feeding on *Meloidogyne* spp. egg masses under *in vitro* conditions (Abou El-Atta *et al.*, 2014).

Sancassania mycophaga (= *Caloglyphus mycophagus*) is a mite that naturally lives in the soil (Hernández-Bacao *et al*., 2015). This species feeds on organic matter, fungi, bacteria and nematodes, facilitating its massive reproduction under laboratory conditions (Chmielewski, 2003). Previous studies have shown that *S. mycophaga* can significantly reduce nematode populations, both parasitic and free-living species. Under *in vitro* conditions, an 81% reduction was found in *Haemonchus contortus* larvae (Strongylida: Trichostrongylidae), a parasitic nematode in sheep, and a complete elimination (100%) of the free-living nematode *Panagrellus redivivus* (Rhabditida: Panagrolaimidae) (Aguilar-Marcelino *et al.*, 2015). Likewise, *S. mycophaga* reduced the population of *Nacobbus aberrans* by 72.9%, and the third-stage larvae of *H. contortus* by 42.17% in laboratory bioassays. Meanwhile, under microplot conditions, reductions reached 78.95 and 63.16% (Quintero-Elena *et al.*, 2022).

This, nematophagous mites represent a potentially efficient tool within integrated management programs for phytoparasitic nematodes (Szewc *et al*., 2021). Nevertheless, additional studies are still needed to validate their effectiveness under field conditions. In this context, this study had the aim of determining the predatory efficiency of *S. mycophaga* against *N. aberrans* under controlled laboratory conditions.

EXPERIMENTAL DEVELOPMENT

Biological Material. Spicy pepper plants (*Capsicum annuum*) cv. California Wonder were used, which were susceptible to *N. aberrans*, and were planted in the Colegio de Postgraduados (COLPOS), Campus Montecillo, State of Mexico, Mexico. The pepper

seeds were disinfested using 1% sodium hypochlorite for 1 minute and then placed on paper towels to induce germination. Once they were germinated, the seedlings were individually transplanted into plastic pots (3.5 cm in diameter and 4 cm in depth) containing 25 cm^3 of fine sterile sand. The pots were kept in a growth chamber at 26 ± 1 °C with a photoperiod of 14:10 h of light: darkness. The plants were fertilized weekly with a nutrient solution that consisted in 3.15 g of Nitrofoska® dissolved in 1 L of sterilized water. The mite, *S. mycophaga*, was obtained from a strain that is kept at room temperature and in the dark (28 ± 2 ºC) in the metabolomics laboratory of the CENID Salud Animal e Inocuidad, INIFAP, Jiutepec, Morelos. The mites were stored in Petri dishes (2 cm in diameter X 1.5 cm in height) containing 5% agar (Hernández-Bacao *et al*., 2015). As a food source, they were provided *P. redivivus* nematodes every seven days from a mixed population of males and females of different ages. The strain *S. mycophaga* was originally isolated in 2009 from soil samples collected in Morelos, Mexico. On the other hand, *N. aberrans* was isolated from galls found in tomato (*Solanum lycopersicum*) roots gathered from experimental plots of the Colegio de Postgraduados, Campus Montecillo, state of Mexico. The nematode eggs were extracted using the tissue disaggregation method described by Vrain (1977) and subsequently incubated in Petri dishes with sterile distilled water at a constant temperature of 28 °C until juvenile hatching. Second-stage (J2) juveniles used in the experiments were five days old at the time of inoculation, ensuring their viability and standardization in the biological tests.

Confrontation of *S. mycophaga* vs. *N. aberrans*. The experiment began when the pepper plants reached the vegetative state with two to three pairs of true leaves. Each experimental unit consisted of a pot with a plant, five adult *S. mycophaga* mites of undetermined sex, and a suspension with a known number of second-stage *N. aberrans* juveniles in accordance with the experiment. The mites were placed by hand on the surface of the substrate using fine entomological forceps, trying not to harm them and allowing them to move immediately in the pot. Two independent experiments were held to evaluate the predatory efficiency of *S. mycophaga* against two densities of the nematode. In the first experiment, 500 J2 were inoculated per experimental unit. Four treatments were evaluated, defined by the moment of inoculation of the nematode in regard to the introduction of the mite (Table 1), with 10 repetitions per treatment. In the second experiment, the treatments were similar, but the nematode density was increased to 1 000 J2 per experimental unit. In this case, only the first three treatments were evaluated (Table 1), with seven repetitions per treatment. The purpose of including two inoculation densities (500 and 1 000 J2) was to evaluate the predatory capacity of *S. mycophaga* under different levels of parasitic pressure, simulating conditions of moderate and high infestation. This strategy helped analyze whether the efficiency of the mite depends on the prey density, which is relevant for its potential use under field conditions.

Table 1. Description of the treatments applied in the confrontation between *S. mycophaga* and *N. aberrans*.

Treatment	Description
T1. Control	Inoculation of nematodes only
T2. SM + NA	Simultaneous inoculation of *S. mycophaga* and J2 of *N. aberrans*
T3. SM + NA-5	Inoculation of J2 of *N. aberrans* five days after the introduction of *S. mycophaga*
T4. SM + NA-10	Inoculation of J2 of *N. aberrans* 10 days after the introduction of *S. mycophaga*

The pots with the pepper plants were placed on plastic lids (5 cm in diameter x 3 cm in height) inside containers with water. The water level was maintained at the level of the

lid to stop mites from escaping. In both assays, a completely randomized experimental design was used.

Estimation of the penetration of nematodes in roots. The penetration of *N. aberrans* was evaluated at days 7, 14 and 21 by destructive sampling: on each day, independent plans were sacrificed for the evaluation. The roots were stained using the sodium hypochlorite-acid fuchsin method, following the technique described by Teillet *et al*. (2013). Subsequently, each root system was examined under a stereoscopic microscope using the 4X and 10X lenses, and the number of J2 juveniles found inside the roots was counted. Based on these counts, the data were transformed into a percentage of individuals that managed to penetrate the root, considering the total amount of J2 inoculated per experimental unit.

Determination of the number of galls and eggs. The number of *N. aberrans* galls and eggs was quantified in seven plants per treatment, exclusively in the experiment with an initial density of 1 000 J2 juveniles. Both variables were expressed per gram of root tissue, and the evaluation was carried out 45 days after inoculation (Figure 1). The egg extraction was carried out following the method described by Vrain (1977). The roots were carefully washed to eliminate substrate residues and stained with lactophenol-acid fuchsin to facilitate the visualization of the nematode structures. Subsequently, a sieving system was used for the recovery of eggs, using sieves with mesh sizes #325 (45 µm) and #500 (25 µm), which helped to efficiently separate the biological material. The quantification was performed using a stereoscopic microscope and the data obtained were standardized according to the weight of the processed root tissue.

Figure 1. A) Root of the bell pepper plant before inoculating with nematodes, **B**) Root with the formation of galls, observed 45 days after inoculating with J2 *N. aberrans* juveniles in the control treatment.

Estimation of the presence of *S. mycophaga*. The presence of *S. mycophaga* was evaluated only in the second experiment, in which 1 000 *N. aberrans* J2 were inoculated per experimental unit. The recovery of mites was carried out on days 14, 21 and 45 after the beginning of the experiment, following the methodology described by Stirling *et al*. (2017), with minor modifications. Every pot was filled with 400 mL of distilled water and shaken by hand for 10 seconds to detach the organisms of the substrate. Later, the content was poured through a sieve with a #200 (75 µm) mesh to hold in the mites. This procedure was performed twice per pot to maximize recovery. Finally, the mites withheld in the mesh were carefully rinsed using a wash bottle and collected in a clean container for subsequent quantification under a stereoscopic microscope.

Statistical analysis. The data corresponding to the percentage of penetration of J2 *N. aberrans* juveniles in the roots were transformed using $\log_{10}(x + 1)$ to comply with the assumptions of normality and homogeneity of variances. The data of the number of galls and eggs per gram of root tissue were transformed using the square root ($\sqrt{x} + 1$). Later, all variables underwent an analysis of variance (ANOVA), and in the cases in which significant differences were found between treatments, a comparison of means was carried out using the Least Significance Difference (LSD) test with a level of significance of $p < 0.05$. The statistical analyses were carried out using the SAS statistical package, version 9.4 (SAS Institute Inc., 2004).

Estimation of the penetration of nematodes in roots. The percentage of penetration of J2 *N. aberrans* juveniles in the pepper roots varied significantly between treatments throughout time (Table 2). In the first 14 days, the presence of the mite *S. mycophaga* did not consistently reduce the invasion of nematodes. In fact, in this period, the treatments with mites presented higher penetration levels in comparison with the control. For example, after 14 days, treatment T2 (simultaneous inoculation of *S. mycophaga* and *N. aberrans*) registered an average of 2.7 nematodes per root, whereas T3 (inoculation of the nematode five days after the mite) reached 6.9, against only 1.3 in the control. However, this tendency changed considerably on day 21. In that moment, all the treatments that included mites displayed a significant reduction in the number of nematodes that penetrated the roots, in comparison with the control. Treatment T2 turned out to be the most efficient one, with an average of only 1.0 nematode per root, against 5.9 in the control. This reduction suggests a delayed but pronounced effect of the mites on the predatory capacity of the J2 juveniles.

In the second experiment, where 1000 J2 were used, a similar dynamic was observed. On day 21, treatments T2 and T3 also displayed a significant reduction in penetration (21.3 and 23.8%, respectively), in contrast with the 41.4% recorded for the control. These results reinforce the hypothesis that *S. mycophaga* can effectively interfere with the establishment of the nematode, particularly after the two first weeks of interaction.

On the other hand, the moment of inoculation of the nematodes in relation with the introduction of the mites was determined to not have had a significant influence on the predatory effect of *S. mycophaga* on the J2, since no statistical differences were found between treatments on day 21.

Table 2. Percentage of penetration of J2 in the root of bell pepper plants in three dates after exposure of *S. mycophaga* vs. *N. aberrans*.

Treatment	Experiment 1[y]			Experiment 2[z]		
	7 days	**14 days**	**21 days**	**7 days**	**14 days**	**21 days**
T1. Control	0.7 ± 0.1c	1.3 ± 0.1bc	5.9 ± 0.2a	1.5 ± 0.2b	28.1 ± 0.8a	41.4 ± 0.3a
T2. Sm + Na	0.5 ± 0.0c	2.7 ± 0.2b	1.0 ± 0.1b	4.0 ± 0.3b	34.5 ± 0.4a	21.3 ± 0.7b
T3. Sm + Na-5	6.5 ± 0.2a	6.9 ± 0.2a	3.4 ± 0.2b	39.1 ± 0.6a	28.4 ± 0.6a	23.8 ± 0.3b
T4. Sm + Na-10	2.2 ± 0.1b	0.1 ± 0.4c	2.5 ± 0.1b	--	--	--

[y]Plants inoculated with 500 J2; [z]Plants inoculated with 1000 J2. Control: Plants only inoculated with *N. aberrans*; Sm + Na: simultaneous inoculation of S. *mycophaga* and *N. aberrans*; Sm + Na-5: inoculation with the nematode 5 days after the addition of the mite; SmNa-10: addition of the nematode 10 days after placing the mite. The values marked with the same letter in each column are not significantly different ($p \leq 0.05$).

Determination of the number of galls and eggs. On day 45 after inoculation with 1 000 J2 *N. aberrans* juveniles, the treatments with and without the presence of *S. mycophaga* displayed no statistical differences in the number of galls or in the number of eggs per gram of root tissue (Table 3). That is, the inclusion of the mite in the treatments did not exert an evident influence on the capacity of the nematode to induce the formation of galls or on its reproduction, at least under de conditions evaluated.

Table 3. Number of galls and eggs per gram of root tissue, 45 days after exposure of *S. mycophaga* with 1 000 *N. aberrans* J2[z].

Treatment	Number of galls	Number of eggs
T1. Control	25.1 ± 0.1a	1 589 ± 0.2a
T2. Sm + Na	25.6 ± 0.1a	2 804 ± 0.1a
T3. Sm + Na-5	21.2 ± 0.03a	1 340 ± 0.2a

[z]The data represent the mean ± standard error. Control: Plants treated only with *N. aberrans*; Sm + Na: Simultaneous inoculation of *S. mycophaga* and *N. aberrans*; Sm + Na-5: inoculation with the nematode 5 days after the addition of the mite. The values marked with the same letter are not significantly different ($p < 0.05$).

The average number of galls per gram of root fluctuated between 21.2 and 25.6, whereas the production of eggs varied between 1 340 and 2 804 per gram of tissue. Treatment T2 (simultaneous inoculation of mite and nematode) recorded the highest number of eggs, followed by the control and T3 (inoculation of the nematode five days after the mite). Despite these numerical variations, the statistical analyses did not identify significant differences between treatments ($p > 0.05$). These results indicate that, under these experimental conditions, *S. mycophaga* did not affect the formation of galls or the oviposition of *N. aberrans*.

Estimation of the presence of *S. mycophaga*. The *S. mycophaga* population increased progressively in the treatments with mites (T2 and T3). On day 14, around 10 individuals were recovered from each pot (≈ +100% than the five initial mites). On day 21, around 25 individuals were quantified (≈ +400%) and on day 45, over 35 (≈ +600%). In the control treatment, no mites were recovered. During the evaluations, eggs, protonymphs, deutonymphs and adults were observed, indicating an establishment and reproduction of the mite in the substrate (Table 4). This sustained growth suggests that the conditions of the experimental system favored the development of the mite, reinforcing its potential as a beneficial organism within an integrated management scheme.

Table 4. Recovery of mites days after exposure of *S. mycophaga* with 1 000 *N. aberrans* J2.

Treatment[z]	14 days	21 days	45 day
T1. Control	0	0	0
T2. Sm + Na	10-15	25-30	35-40
T3. Sm + Na-5	10-15	25-30	35-40

[z]Control: Plants treated only with *N. aberrans*. Sm + Na: simultaneous inoculation of *S. mycophaga* and *N. aberrans*. Sm + Na-5: inoculation of the nematode five days after the mite. The values presented represent approximate ranges per pot.

The effectiveness of the nematophagous mites to reduce nematode populations depends on several factors, some of the most outstanding of which are the density of released mites and the availability of prey (Xu *et al*., 2014; Yang *et al*., 2020). Some studies have proven that certain predators increase their rate of intake as the amount of available prey increases (Xu *et al.,* 2014). Nevertheless, in this study, la depredation exerted by *S. mycophaga* on *N. aberrans* J2 showed no significant variation between treatments with different nematode densities (500 and 1000 J2), suggesting that the predatory behavior of this mite may be independent to the initial amount of prey offered. Yang *et al.* (2020) indicated that the optimum density for the control of *Meloidogyne incognita* with *Stratiolaelaps scimitus* was 400 mites per pot. In contrast, in this study, only five *S. mycophaga* individuals were introduced per pot. Despite this low initial density, a significant reduction was found in the number of nematodes that penetrated the roots in comparison with the control, although this effect was only evident starting on day 21 after the inoculation of the nematode. This delay in the efficacy can be explained by the time required for the mites to adapt, reproduce and increase their population. Likewise, it is likely that *N. aberrans*, in different phases of its life cycle, was exposed to active predation by *S. mycophaga*, since this nematode species enters and exits the host roots during its development, until the immature females establish a permanent feeding site and become sedentary (Stirling, 1991; Manzanilla-López *et al*., 2002; Eves-Van den Akker *et al*., 2014). This feature of the *N. aberrans* life cycle could have favored the interaction with predatory mites for longer, thus increasing the opportunities for predation. Stirling *et al*. (2017) reported a greater percentage of predation of *Protogamasellus mica* over *Tylenchorhynchus annulatus* in comparison with *Pratylenchus zeae* in sugarcane (*Saccharum officinarum*) plants, suggesting that this difference is due to the feeding habits of *T. annulatus*. When it feeds, the head of *T. annulatus* remains inside the roots, while its body is exposed, making it vulnerable to predation by *P. mica*.

The interaction between predator and prey can be influenced by factors such as the environment, the time for which the predator starves and the ambient temperature (Yang *et al*., 2020). The new and unusual environment to which both *S. mycophaga* and the *N. aberrans* J2 were exposed may have imposed pressure affecting both species, given that both were adapted to laboratory conditions (Wharton, 2004; Gang and Hallem, 2016).

The number of *N. aberrans* galls and eggs found in the roots of treated plants displayed no significant differences between the different treatments and the control. Similarly, Xu *et al*. (2014) found that the number of galls and the egg masses produced by *Meloidogyne incognita* in *Ipomoea acuatica* plants did not vary between the treatment with the nematophagous mite *Blattisocius dolichus* and the control. These finding suggested that, under certain conditions, the predatory mites may not directly affect the oviposition or formation of galls, at least in the early phases of the plant-nematode-mite system. In several organisms, females display defensive behaviors as a strategy to increase the survival of their offspring (Saitoh *et al*., 2021). In this context, *N. aberrans* could display a similar behavior to safeguard its eggs from the biotic stress produced by predators (Palacios-Alcántara *et al*., 2022).

Although this study found no significant differences in the number of galls or eggs between treatments, a progressive reduction was achieved in the amount of J2 juveniles that penetrated the roots throughout time in the treatments with *S. mycophaga*. These results reinforce the hypothesis that *S. mycophaga* has a promising potential as a biological control agent for *N. aberrans*. However, it is necessary to carry out additional investigations that

include different mite densities, long-term evaluations and tests under field conditions. In this sense, biological characteristics such as their short life cycle, its small size and its parthenogenetic reproductive capacity confers significant advantages to *S. mycophaga*, which position it as a viable candidate for inclusion in integrated management programs for phytoparasitic nematodes (Al-Ani *et al*., 2020).

CONCLUSIONS

S. mycophaga reduced the penetration of *N. aberrans* in pepper plant roots. After 21 days, with 500 J2, penetration fell from 5.9 J2 per plant in the control to 1.0–3.4 J2 in the treatments with mites (relative reduction of 42–83%). With 1000 J2, penetration dropped from 41.4% in the control to 21.3–23.8% (42–49%). Mite population increased from five initial individuals to ~10, ~25 and ~35 mites per pot on days 14, 21 and 45, respectively, confirming its establishment and reproduction in the substrate. These results indicate that *S. mycophaga* has the potential to be used as a biological agent in the management of *N. aberrans*.

Conflict of interest. The authors declare there are no conflicts of interest related to this study.

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Contribution of the authors. Olga Gómez-Rodríguez: Conception of the study, design and execution of the experiment, preparation of the manuscript; **Edgar Villar-Luna:** design and execution of the experiment, preparation of the manuscript; **Laith Khalil Tawfeeq Al-Ani:** Analysis and interpretation of data, statistical analysis; **Iván Morales-Soto:** Analysis and interpretation of data; **Reyna Vargas-Abasolo:** Editing and revision of the manuscript; **Liliana Aguilar-Marcelino:** Conception of the study and approval of the final version of the manuscript.

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