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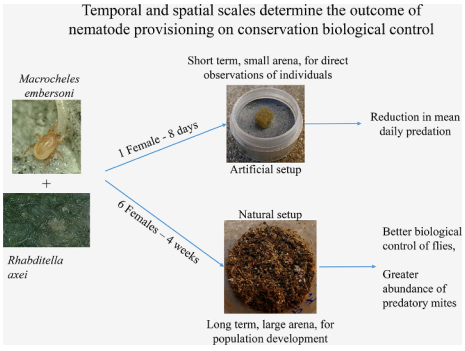
Free living nematodes as alternative prey for soil predatory mites: An interdisciplinary case study of conservation biological control

L.H. Azevedo[a], L.G. Leite[b], J.G. Chacon-Orozco[b], M.F.P. Moreira[a], M.P. Ferreira[a], L.M. González-Cano[a], V. Borges[a], D. Rueda-Ramírez[a,d], G.J de Moraes[a], E. Palevsky[c,*]

[a] Departamento de Entomologia, Escola Superior de Agricultura "Luiz de Queiroz" (ESALQ), Universidade de São Paulo, Piracicaba, SP, Brazil
[b] Instituto Biológico (IB), Campinas, SP, Brazil
[c] Newe Yaar Research Center, Agricultural Research Organization (ARO), Israel
[d] Facultad de Ciencias Agrarias, Universidad Nacional de Colombia, Bogotá, Colombia

GRAPHICAL ABSTRACT



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ABSTRACT

Species of soil predatory mites feed on a diverse diet making them excellent biocontrol candidates for conservation biocontrol programs. Free-living nematodes (FLN) are commonly found in soils and serve as prey for many soil predatory mites, but as far as we know, have never been used as alternative prey to enhance the efficacy of soil predatory mites for conservation biological control. Our goal in this case study was to determine whether the FLN *Rhabditella axei*, provisioned as complementary prey, would improve the efficacy of *Macrocheles embersoni* as a biocontrol agent of the housefly *Musca domestica*. Two experimental setups differing temporally and spatially were conducted. The first, performed in small Petri dish arenas over 10 days, assessed *M. embersoni* fecundity and predation of L1 *M. domestica*, with or without supplementation of *R. axei*. The second, carried out in plastic containers over four weeks, was provisioned three times a week with *M. domestica* eggs and fresh larva diet, with or without nematode supplementation. The efficacy of fly immature predation was estimated by counting the adult flies that emerged. In the short-term, small arena, experiment, nematode supplementation reduced predation. Similarly, in the long-term experiment in plastic containers, more flies emerged in the nematode supplemented treatment during the 3rd week (the 1st week of fly emergence). However, in the 4th week, fly emergence dropped dramatically in the nematode supplemented treatment, whereas fly emergence continued to escalate in the

* Corresponding author.
E-mail address: palevsky@volcani.agri.gov.il (E. Palevsky).

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treatment that received only fly eggs, and *M. embersoni* abundance was about a third of that in the nematode supplemented treatment. In summary, complementing the diet of *M. embersoni* with nematodes resulted in higher predator abundance and better biological control.

1. Introduction

Many species of predatory mites feed on a diverse diet, making them excellent candidates for conservation biological control programs (Carrillo et al., 2015). Such programs have also benefited from provisioning supplemental food for these natural enemies to boost predator abundances and maintain predator populations during low prey abundance periods. Pollen, for example, provisioned in small containers (Nomikou et al., 2010), dusted on plants (Nomikou et al., 2002), applied with blowers (Pijnakker et al., 2016) and released from hedge rows (Duso et al., 2004) or cover crops (Maoz et al., 2011; Smith and Papacek, 1991; Warburg et al., 2019) has improved the conservation of phytoseiid (Mesostigmata: Phytoseiidae) mites in biological control scenarios. Likewise, sachets containing species of the mite cohort Astigmatina (O'Connor, 2009), considered factitious prey, and their respective diet have been developed as open rearing units for several phytoseiid species to support the control of greenhouse pests (Calvo et al., 2015; Sampson, 1998; Shipp and Wang, 2003).

Many successful conservation biological control studies have been conducted utilizing predatory mites, especially phytoseiids (Gerson et al., 2003; Messelink et al., 2014), for the control of above ground pests. However, comparatively few studies have investigated predatory mites for conservation biological control on or below ground. Astigmatina have recently been reported as factitious prey for rearing predatory mesostigmatid soil mites of the families Laelapidae and Rhodacaridae (Barbosa and Moraes, 2016). They have also been used as alternative prey to conserve populations of *Macrocheles robustulus* (Berlese) (Mesostigmata: Macrochelidae) to enhance the biological control of sciarid flies (Grosman et al., 2011) and for *Cosmolaelaps* n. sp. (Mesostigmata: Laelapidae) for the control of the prepupae and pupae of the western flower thrips (WFT) *Frankliniella occidentalis* (Pergande) (Munoz Cardenas, 2017). The use of astigmatine species as factitious prey is a very cost effective solution for rearing and releasing predators for augmentative biocontrol in intensive cropping systems such as greenhouses and screen houses. However, they may not be the ideal choice for conservation biological control in the soil. Astigmatina were likely utilized in the above studies due to ease of access, since they are readily available from biocontrol companies, as opposed to ecological sense. Alternatively, natural prey may provide a more effective source of alternative food for soil predatory mites, but it remains unclear whether specific prey may be commonly utilized by several acarine soil predator families.

Free living non parasitic nematodes (FLN) are found in soils (Neher, 2010) and serve as prey for many insects and soil mites (Heidemann et al., 2014). Some species of mites (i.e. Ascidae and Macrochelidae) are so reliant on nematodes as prey that they did not lay eggs without first consuming nematodes (Walter et al., 1987), while others experience increased fecundity when nematodes are available. For example, the fecundity of *Lasioseius floridensis* Berlese (Mesostigmata: Blattisociidae) was 3 fold higher when fed the FLN *Rhabditella axei* (Cobbold) rather than *Tyrophagus putrescentiae* (Schrank) (Astigmatina: Acaridae) (Britto et al., 2012). Similarly, fecundity of *Cosmolaelaps jaboticabalensis* Moreira, Klompen and Moraes (Mesostigmata: Laelapidae) and *Macrocheles embersoni* Azevedo, Berto and Castilho were highest when fed *R. axei* (Azevedo et al., 2018; Moreira et al., 2015). Food preference, however, differs among life stages of particular predatory mites. *Macrocheles muscaedomesticae* (Scopoli) nymphs were shown to prefer FLN over housefly (*Musca domestica* L.) eggs, while the opposite was true for adults (Rodriguez et al., 1962). Immatures of *Parasitus bituberosus* Karg were also found to be reliant on FLN to complete development to an adult stage (Rueda-Ramírez et al., 2019). Surprisingly, as far as we know, FLNs have never been used as alternative prey to enhance the efficacy of soil predatory mites for the conservation biological control of soil pests.

Our goal in this case study was to determine whether *R. axei* could be used to enhance *M. embersoni* conservation biological control of the housefly. This relatively simplified system was chosen as a model because it was recently used to evaluate the biological potential of *Macrocheles* species for fly biocontrol (Azevedo et al., 2018). In this study as well as others (Britto et al., 2012; Moreira et al., 2015), *R. axei* was reared on decaying bean and provisioned in surplus in liquid, containing the decaying bean and its associated microbial community. In these rearing units and experimental arenas (also used in the present study), nematodes were clearly seen swimming under a biofilm. We anticipated that the components of this system might be important in utilizing FLN in conservation biological. Accordingly, in the present study, we address the following research questions: Will the provisioning of FLN enhance predator fecundity in small arenas in short term experiments and predator abundance in large arenas in long term experiment? Could the nematode diet diversity and dose, affect the fecundity and population growth of predatory mites? What is the role of the biofilm in this system? Finally, will supplementation of FLN increase biocontrol efficacy?

We hypothesized that provisioning with *R. axei* could reduce fly larvae predation in small arenas under short time periods, possibly due to satiation of the predator. Conversely, we hypothesized that in larger arenas and over a longer time period nematode provisioning would enhance the biocontrol of the housefly, as a diverse prey diet would support a significantly higher predator population growth (Messelink et al., 2010).

2. Methods & materials

This study was conducted between December 2017 and August 2018 at the Department of Entomology and Acarology, ESALQ, Piracicaba, University of São Paulo, SP, Brazil. Experiments were conducted in incubators in darkness, in experimental units consisting of small Petri dish arenas (2.7 cm in diameter and 1.2 cm high), at 28.0 ± 0.3 °C, 90 ± 5% RH (experiments 2.1.1 and 2.1.2) and of large clear plastic container arenas (200 cc) at 25.4 ± 0.3 °C, 79 ± 2% RH (experiments 2.2.1 and 2.2.2), herein referred to as small and large arenas. Video clips were taken with a 3D digital microscope (Hirox KH-8700, http://www.hirox.com) using the rotary head and for one clip in macro, using the digital single lens mirrorless (DSLM) camera (Panasonic model DMC-FZ300, https://www.panasonic.com). Videos were edited and formatted to MP4 using iMovie (https://www.apple.com/lae/imovie/) and uploaded as video files to the journal website. Video clips were taken primarily to capture the behaviors of *R. axei* and *M. embersoni* in the different setups (rearing and experimental) used in this study.

Macrocheles embersoni were collected from laying hens droppings at Jaboticabal, in São Paulo State, Brazil, in September 2017 and subsequently reared at ambient temperature in plastic containers (12 cm diameter and 7.5 cm high), filled to 70% of its volume with vermiculite. To maintain humidity, distilled water was added once a week to the container's plaster floor, made of a mixture of gypsum powder and activated charcoal, at a ratio of 9:1 (Abbatiello, 1965). *Macrocheles embersoni* were fed three times a week with a mixture of all stages of *R. axei* and with eggs and larvae of the housefly. Colonies of *R. axei* were maintained in plastic containers (10 cm diameter and 6 cm high) with

rotting pieces of pods of *Phaseolus vulgaris* L. (green bean) soaked in distilled water. Fresh pieces of pods were added three times a week. In these rearing units we readily observed masses of *R. axei* swimming below a biofilm, considered to be secreted by the decomposing microbial community (Video Clip 1). Larvae of houseflies were maintained on a diet composed of wheat bran (1180 ml), dried alfalfa (475 ml) ground in a blender, oats (240 ml), malt (3.5 g) and brewer's yeast (3.5 g) mixed with 1.1 L of water and set to stand at room temperature for 24 h before use (amounts indicated yields approximately 3 L of diet). Fly adults were maintained on a mixture of milk (100 ml) and sugar (1 g).

2.1. Small arena experiments

2.1.1. Effects of FLN on predation and fecundity of Macrocheles embersoni in small arenas

For ten days, we recorded L1 *M. domestica* predation and *M. embersoni* fecundity. Prey treatments, provisioned daily, consisted of: 1) 20 L1 *M. domestica* + 50 µl of water; 2) 20 L1 *M. domestica* + 200 *R. axei* in 50 µl of decomposing bean medium. Effects on fecundity included a third treatment of only 200 *R. axei* in 50 µl of decomposing bean medium. Each small arena (experimental unit) contained one gravid, 0–2 days old female of *M. embersoni* (Azevedo et al., 2018), taken from the laboratory colony described above (in total, 3 prey treatments * 10 replicates = 30 units). Arenas were sealed with clear plastic film (Magipack®, Felix Pack, São Paulo, Brazil). Once a day, L1 fly larvae were replenished and either 50 µl of decomposing bean with *R. axei* in surplus or water were added with a micropipette to a 9 mm cube of green foam (phenolic foam substrate for germination and rooting, https://www.floralatlanta.com.br/, São Paulo, Brazil) placed in the middle of the arena. As the foam deteriorated over time due to burrowing fly larvae, it was replaced on day six. Predation of fly larvae was recorded by counting the number of dead L1 housefly. Because *M. embersoni* is known to be cannibalistic and the egg stage at 25 °C lasts less than 1 day (Azevedo et al., 2018), fecundity was determined by counting and removing the eggs and larvae twice a day (in the morning and afternoon). Mean predation and fecundity were calculated from day three to minimize the diet effect of the pre-experimental period. ANOVAs were used to determine effects of diet on fly predation and predator fecundity and the post hoc Tukey's Test for analyzing differences between means. To evaluate the prevalence of biofilm, we recorded throughout the experiment, in the morning and afternoon, the biofilm status as intact, partially intact and not visible. Additionally, we noted nematode exposure as exposed or not exposed. We assumed that biofilm intactness and nematode exposure would be affected by nematode provisioning, with fewer arenas with intact biofilm and more arenas with exposed nematodes in the afternoon, 8 h after nematode provisioning than in the morning, 16 h post provisioning. Chi Square tests were utilized to test these hypotheses.

2.1.2. FLN dose and rearing-diet effects on Macrocheles embersoni fecundity in small arenas

Using the same experimental unit described in Section 2.1.1, the effect of nematode dose and rearing-diet on *M. embersoni* fecundity was observed twice a day over a period of eleven days, replicated five times. Four nematode doses, 0, 50, 200 and 1000 reared on either decomposing bean or baker's yeast *Saccharomyces cerevisiae* cultured on nutrient agar (https://www.sigmaaldrich.com) were compared (in total, 4*2*5 replicates = 40 small arenas, with one gravid). Nematodes were provisioned in a liquid volume of 400 µl to a 6 mm cube of green foam (determined empirically as the maximum volume of liquid this sponge size could adsorb) placed in the center of the arena. Cubes were replaced daily to facilitate recording and removal of *M. embersoni* eggs and larvae. In this experiment, to absorb excess moisture from the foam on one hand but to retain humidity on the other, the Petri dish floor was punctured 4 times with a hot dissecting needle and coated with plaster as described above for the *M. embersoni* rearing container (Fig. 1). To obtain the desired doses, counts were performed under a light microscope at 10x in a precision-chambered counting slide (http://astelbtu.com.br/lamina-para-contagem-de-nematoides-mod-m-metalizada.html) for each nematode rearing-diet. Accordingly, dilutions were performed. As in the first experiment, we used fecundity values from day three onwards. Non-linear logarithmic regression was used to test the effect of dose as a continuous variable on fecundity for each diet. As there was no significant difference within each diet between doses 200 and 1000, diets were compared using the pooled data (for doses 200 and 1000) and subjected to Wilcox/ Kruskal-Wallis, as ANOVA assumptions were not met.

2.2. Large arena experiments

2.2.1. FLN diet effects on Macrocheles embersoni population growth in large arenas in vermiculite

Population growth of *M. embersoni* provisioned with nematodes reared on one of the two diets, decomposing bean or yeast, replicated five times (in total, 2*5 = 10 units), was recorded after ten days. The large arena, was filled with 125 cc of humidified vermiculite with openings (3*2 cm) on the bottom and lid, covered with nylon mesh (100 µm pore size), glued on with a hot melt gun. Vermiculite was kept in an oven for 24 h at 60° to kill any potential contaminants. Then to humidify the vermiculite, water was slowly added to the vermiculite, in a mixing bowl, at a ratio of 1:4, the day before beginning the experiment. To absorb excess water, an additional part of vermiculite was added and mixed thoroughly (bringing the water vermiculite ratio to 1:5). To avoid compaction, vermiculite was carefully spooned into the experimental unit. Six gravid females of *M. embersoni* were placed in each container and these were fed with daily applications of 2000 nematodes in a volume of 1 ml. After 10 days, the vermiculite from each experimental unit was spread on a large piece of white filter paper and the number of walking predators visible to the naked eye were counted. A t-Test was used to determine the rearing-diet effect on population growth.

2.2.2. Effects of predator and FLN on housefly emergence and predator population growth in large arenas in fly larvae culture medium

We evaluated the effects of *M. embersoni* (with and without predators) and nematode provisioning (with and without nematodes), on

Fig. 1. Small experimental arena. Nematodes were provisioned in a liquid volume of 400 µl to the green foam (phenolic foam substrate for germination and rooting, https://www.floralatlanta.com.br/, São Paulo, Brazil). Plaster floor made of a mixture of gypsum powder and activated charcoal, at a ratio of 9:1 (Abbatiello, 1965).

fly emergence, replicated six times (in total, 2*2*6 = 24 units). With the initiation of the experiment, 5 g of housefly larvae diet, 30 fly eggs, 3000 nematodes (reared on decomposing bean) and six gravid females of *M. embersoni* (to the respective predator and nematode treatments) were added to each ventilated large arena (described in Section 2.2.1). For the duration of the experiment, three times a week, we continued to provision the same amounts of housefly larvae diet, fly eggs and nematodes (to the respective treatment).

Following the commencement of fly emergence (two weeks into the experiment), emergence was recorded three times a week for two weeks. The experiment ended after four weeks. For the extraction of *M. embersoni*, the contents of each arena was poured into a modified Berlese funnel and processed for one week. The effect of nematode provisioning on predator abundance was analyzed with the Wilcox/Kruskal-Wallis test, as ANOVA assumptions were not met. Total fly emergence per week was analyzed with a linear mixed effects model (package lme4 of R, version 3.4.4, The R foundation for Statistical Computing, 2018-03-15) with replicate (arena) as random factor and predator (2 levels), nematodes (2 levels) and time (2 levels) as fixed factors. The significance of factors and their interaction was determined by comparing models with and without them with the ANOVA function of R.

3. Results

3.1. Small arena experiments

In small arenas, supplementation of *R. axei* significantly reduced *M. embersoni* predation of *M. domestica*, by about 3 larvae per day (Table 1a). However, this supplementation did not enhance fecundity as daily means were similar when fed L1 *M. domestica*, *R. axei* and their combination. In the 400 observations conducted during the 10-day experiment, biofilm status was either mostly intact (77%) or partially intact (23%), but there were no cases when biofilm was not present. Hours from nematode provisioning (Table 1b) did not significantly affect biofilm intactness but significantly more nematodes were exposed in the afternoon (8 h from nematode provisioning) (Video Clip 2).

Non-linear logarithmic regression (Fig. 2) revealed a significant dose effect of nematodes reared on bean on the mean daily fecundity of *M. embersoni* (Fecundity = 0.0025 + 0.099Ln(Dose + 1), $R^2 = 0.69$, P = 0.0000054), whereas nematodes reared on yeast had no effect (Fecundity = 0.04 + 0.014Ln(Dose + 1), $R^2 = 0.11$, P = 0.1486). The effect of nematode diet on fecundity of *M. embersoni* (for the combined data set for doses 200 and 1000) was highly significant (P = 0.0002) with mean daily fecundity values on bean being six times that on yeast.

3.2. Large arena experiments

Nematode rearing diet significantly affected population growth of *M. embersoni* (P = 0.0002) over ten days in large arenas in humidified vermiculite. Mean number (± SE) of *M. embersoni* motiles was three times higher (14.4 ± 1.12 mites/container) when fed nematodes reared on bean compared to predators provisioned with nematodes reared on yeast (4.8 ± 0.73 mites/container).

Fly emergence was significantly lower with *M. embersoni* than without ($X^2 = 7.45$, D.f. = 1, P = 0.006). The interaction of week and nematode supplementation on fly emergence was very significant ($X^2 = 10.26$, D.f. = 1, P = 0.001), indicating that the effect of nematode supplementation on fly biocontrol changed in the second week.

In the first week of fly emergence, fly biocontrol was significantly better with predators than without predators (P = 0.029), especially when nematodes were not provisioned (P = 0.020) (Fig. 3). However, predators with nematode supplementation fed on fewer flies, resulting in higher fly emergence, in arenas with than in arenas without nematodes ($X^2 = 59.524$, D.f. = 1, $P = 1.2 \times 10^{-14}$). Fly emergence with nematode provisioning, was similar with and without predators (P = 0.186).

In contrast, in the second week of fly emergence, predators with nematode supplementation significantly reduced fly emergence compared to predators without nematode provisioning (P = 0.012). Fly emergence in the predator treatment was lower than the no predator treatment with nematode provisioning (P = 0.014), but it was similar with or without predators when nematodes were not provisioned. Mean number (± SE) of predators extracted from the fly larvae medium following the second week of fly emergence was almost three times higher in the nematode provisioning treatment than in the control (1143 ± 171 vs. 431 ± 208; P = 0.045).

4. Discussion

As hypothesized, supplementing with nematodes, as an additional food source in the small arena trial, significantly lowered *M. embersoni* predation of L1 *M. domestica*. This could be attributed to satiation, as *M. embersoni* fed both on L1 *M. domestica* and on *R. axei* (Video Clip 3). A comparable decrease in pest predation in small arenas was reported for the soil mite *Gaeolaelaps aculeifer* (Canestrini) (Mesostigmata: Laelapidae) when the astigmatine mite *Aleuroglyphus ovatus* (Troupeau) was offered together with the WFT (Rueda-Ramírez et al., 2018). Similarly, on leaf disc arenas, in the presence of pollen as supplementary food, *Iphiseius degenerans* (Berlese) (Mesostigmata: Phytoseiidae) preyed on fewer citrus rust mites (CRM) *Phyllocoptruta oleivora* (Ashmead) (Prostigmata: Eriophyidae) than when offered CRM only (Palevsky et al., 2003). The transient negative effect of nematode provisioning on biocontrol in the first week of fly emergence was similar to the result of our small arena larval predation experiment. Predators at an initially low density and an abundance of food may become satiated, or alter their functional response to fly prey, consistent with apparent mutualism. Subsequently, in the second week, nematode provisioning significantly enhanced biocontrol (reduced fly emergence). We attribute this to accelerated growth of the provisioned predator population, compared to non-provisioned controls, resulting in increased predation pressure on both prey species, consistent with apparent competition (Holt and Bonsall, 2017).

While mean fecundity of *M. embersoni* in the present study was slightly higher in the arenas with both FLN and L1 *M. domestica*, the difference was not significant. Fecundity of *G. aculeifer* did not differ when offered WFT or WFT plus *A. ovatus* (Rueda-Ramírez et al., 2018). In contrast, supplementing the diet of western flower thrips with *R. axei* almost doubled the fecundity of *P. bituberosus* (Rueda-Ramírez et al., 2019). Mean fecundity levels found in the present study on L1 *M. domestica* and *R. axei* (both approximately 1 egg/day) were substantially lower than those reported by Azevedo et al. (2018) (3.6 and 5.4 eggs/day, respectively). We assumed that eggs laid in the skeleton of the green foam in this study went undetected. To confirm this assumption, we setup up additional identical arenas and carefully cut the foam four days later under a dissecting microscope. Inside the foam skeleton we observed eggs, immatures and adults of *M. embersoni*, as well as *R. axei*

Table 1a
Mean ± SE daily larval predation of L1 *Musca domestica* and fecundity of *Macrocheles embersoni* in small arenas with and without the provisioning of the free living nematode *Rhabditella axei* (as an additional food source) and fecundity of *M. embersoni* when offered only *R. axei*. Different lower case letters indicate a significant difference between prey treatments, *P* values for predation T test < 0.0001 and for Fecundity F test 0.43; n = 10.

	L1 *M. domestica* Predation		*M. embersoni* fecundity	
Prey	Mean	SE	Mean	SE
M. domestica	17.8 a	0.30	1.1 a	0.10
M. domestica + *R. axei*	14. 9 b	0.37	1.3 a	0.17
R. axei			1.0 a	0.14

Table 1b
Proportion (%) of arenas with intact biofilm and exposed nematodes, in the morning and afternoon (sixteen and eight hours after nematode provisioning, respectively). Different lower case letters indicate a significant difference. P values for Chi Square tests for effects on biofilm intactness and exposed nematodes were 0.07 and < 0.0001, respectively, n = 200.

Hours after nematode provisioning	Bio film Intact	Nematodes exposed
16 h (morning)	73.5 a	43.0 b
8 h (afternoon)	81.0 a	70.0 a

(Video Clip 4). While this could be problematic, depending on the bioassay, the advantage of utilizing the green foam was that we could apply a designated volume of nematodes in liquid form, without the nematodes desiccating. This led to very significant effects of FLN provisioning in the small arena experiments, on predation, as well as on fecundity in the diet and dose experiment.

The proportion of exposed nematodes dropped by almost 30% between 8 and 16 h after application, apparently by finding their way into the green foam. The prevalence of a visible biofilm on the cube of green foam, after applying only 50 µl of decaying bean medium per day, whether intact (77%) or partially intact, in all 400 observations is worth noting. Biofilm is an extracellular matrix produced by living multicellular bacterial communities (López et al., 2010). For marine FLN, biofilms can serve as a food source, provide protection from desiccation and be utilized for structure and shelter (Claudia et al., 2004). Biofilms secreted by non-pathogenic bacteria can also induce stress resistance and prolong FLN lifespan (Smolentseva et al., 2017). The role played by the biofilm in our 'bean soup' rearing units kept at ambient temperature and RH and in non-sterilized conditions has yet to be determined. What is of interest to note is that nematode abundance in these open rearing units remained stable and lacked any visible disrupting contaminants by fungi, indicating that the bean soup may contain antifungal compounds.

The reason for significantly higher *M. embersoni* fecundity levels attained on nematodes reared on decomposing bean compared to nematodes reared on yeast in a medium of nutrient agar in small arenas and population growth in large arenas merits further study. It could be due to an indirect effect of these diets on *M. embersoni*, by affecting nematode abundance, or to a direct effect, by the ingestion of the gut content of the nematodes or both. If the latter, FLN could be used to assess various diets on predatory mite fitness, providing these diets would meet the dietary requirements of the respective nematodes. Studies with phytoseiids have shown that supplementing the diet of their prey can enhance both prey and predatory mite populations. The addition of yeast powder, sugar and glucose to the diet of *T. putrescentiae* increased fecundity and shortened duration of development of *Neoseiulus barkeri* Hughes (Huang et al., 2013). On chrysanthemums, the combined release of *Amblyseius swirskii* Athias-Henriot with the astigmatine mite *Carpoglyphus lactis* (L) plus diet 'A' compared to the same combination without diet 'A' resulted in higher populations of *C. lactis* and *A. swirskii* (Hoogerbrugge et al., 2008).

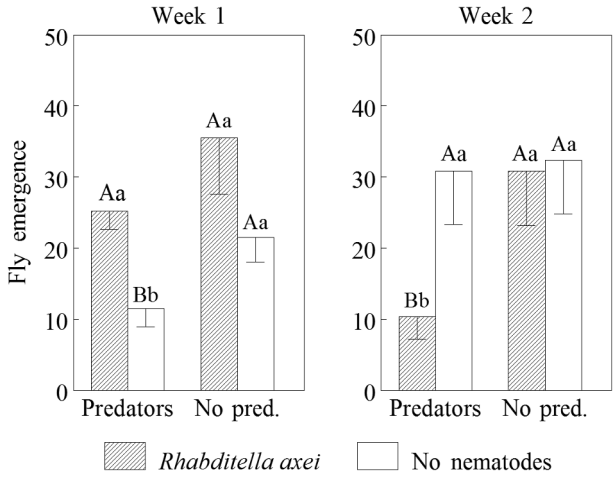

Fig. 3. Effects of the soil mite predator *Macrocheles embersoni* and the free living nematode *Rhabditella axei* on the mean number of flies that emerged in the first and second week of fly emergence (note: fly emergence began in the third week of the experiment). Different uppercase letters indicate a significant predator effect (α = 0.05) within each nematode treatment for each week. Likewise, different lowercase letters indicate a significant nematode effect (α = 0.05) within each predator treatment.

In our small arena experiment, we recorded fecundity and predation by directly counting the number of eggs laid and L1 *M. domestica* killed, respectively. In our larger arena experiment, over a period of several weeks, we indirectly assessed the biocontrol of fly immature life stages by counting the fly adults that emerged. While the latter experimental setup cannot yield detailed information on fecundity or predation of a life stage, it does have several advantages over the small arena setup pertaining to its relevance to real conditions and potential for monitoring population growth. The fresh fly larvae diet added periodically as a food source for the immature flies decomposed to organic matter and was actually quite similar to the natural litter environment of soil predatory mites and nematodes (Thoden et al., 2011). Interestingly, the borrowing of the fly larvae had a dramatic effect on the 'litter' structure of this medium (Video Clip 5), apparently facilitating the movement of *M. embersoni*. Mesostigmatid mites are usually negatively phototropic

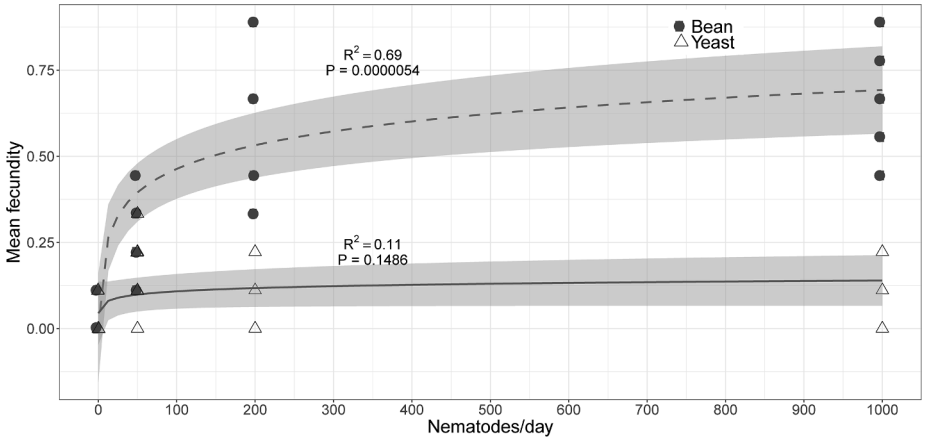

Fig. 2. Mean daily fecundity of *Macrocheles embersoni* provisioned once a day with 0, 50, 200 and 1000 nematodes reared on either yeast or decomposing bean. Non-linear regression lines for *M. embersoni* provisioned with nematodes reared on bean (P = 0.0000054) and yeast (P = 0. 1486).

(Tachi and Osakabe, 2012; Weintraub et al., 2007). *Macrocheles embersoni* in small arenas exposed to the lighting of the 3D digital microscope (used for taking the video clips) were hyperactive, stressed and sought out holes in the plaster to hide. In contrast, in the larger arenas *M. embersoni* moved freely into the litter-like decomposing fly medium, effectively escaping the stress induced by direct light exposure. The range of moisture content in this medium can accommodate the moist to arid requirements of the predatory mite and the relatively wet environment more suitable to the free living nematodes (Video Clip 6). Under these relatively natural conditions, with nematode provisioning, the predator populations developed from 6 to a mean of over 1100 mites in 4 weeks.

We have conducted our case study on *M. domestica*, *M. embersoni* and *R.* axei, all three organisms found interacting under natural conditions. *Macrocheles embersoni* feeds on fly eggs and first instar larvae (Azevedo et al., 2018) and *Macrocheles* females are known to be phoretic on flies (Faryish and Axtell, 1971; Rodrigueiro and Prado, 2004). Species of Rhabditidae are phoretic on flies (Rinker and Bloom, 1982) and *Macrocheles* species (Flechtmann et al., 1980) (Video Clip 7), the latter feeding on *R. axei*. For this specific food web we have demonstrated that the FLN *R. axei* can be used to conserve and augment the soil predatory mite *M. embersoni* and enhance biocontrol of the housefly.

Further studies are needed for the identification and conservation of FLN species that could be utilized for the conservation of soil predators to foster the control of plant soil pests. In outdoor crops, manipulations of soil management such as irrigation in the dry season and organic amendments have been used to enhance populations of naturally occurring FLN for nitrogen mineralization and disease management (Bulluck et al., 2002; Ferris et al., 2004; Rahman et al., 2014; Thoden et al., 2011). Similar soil manipulations could be used for identifying indigenous communities of FLNs and soil predatory mites in agricultural plots and their surrounding natural environments. This could serve as a starting point for evaluating species of FLNs and predatory mites for the control of specific soil pests. For short-term evaluations an interesting experimental setup could be the green foam (used in the present study in our small arena trials) as both the FLN and predatory mites readily established within the foam skeleton. It could also be used to screen plant growth promoting bacteria for plant fitness and population growth of FLN (Kimpinski and Sturz, 1996).

CRediT authorship contribution statement

L.H. Azevedo: Funding acquisition, Supervision, Conceptualization, Writing - review & editing. **L.G. Leite:** Methodology, Conceptualization, Writing - review & editing. **J.G. Chacon-Orozco:** Methodology, Conceptualization, Writing - review & editing. **M.F.P. Moreira:** Methodology, Conceptualization, Writing - review & editing. **M.P. Ferreira:** Methodology, Visualization, Conceptualization, Writing - review & editing. **L.M. González-Cano:** Methodology, Conceptualization, Writing - review & editing. **V. Borges:** Methodology, Conceptualization, Writing - review & editing. **D. Rueda-Ramírez:** Formal analysis, Conceptualization, Writing - review & editing. **G.J de Moraes:** Conceptualization, Project administration, Writing - review & editing. **E. Palevsky:** Conceptualization, Funding acquisition, Writing - original draft, Writing - review & editing.

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Appendix A. Supplementary data

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References

Abbatiello, M., 1965. A culture chamber for rearing soil mites. Turtox News 43, 162–164.
Azevedo, L.H., Ferreira, M.P., de Campos Castilho, R., Cançado, P.H.D., de Moraes, G.J., 2018. Potential of *Macrocheles* species (Acari: Mesostigmata: Macrochelidae) as control agents of harmful flies (Diptera) and biology of *Macrocheles embersoni* Azevedo, Castilho and Berto on *Stomoxys calcitrans* (L.) and *Musca domestica* L. (Diptera: Muscidae). Biol. Control 128, 1–8.
Barbosa, M.F., Moraes, G.J., 2016. Potential of astigmatid mites (Acari: Astigmatina) as prey for rearing edaphic predatory mites of the families Laelapidae and Rhodacaridae (Acari: Mesostigmata). Exp. Appl. Acarol. 69, 289–296.
Britto, E.P.J., Gago, E., Moraes, G.J., 2012. How promising is *Lasioseius floridensis* as a control agent of *Polyphagotarsonemus latus*? Exp. Appl. Acarol. 56, 221–231.
Bulluck, L., Barker, K., Ristaino, J., 2002. Influences of organic and synthetic soil fertility amendments on nematode trophic groups and community dynamics under tomatoes. Appl. Soil Ecol. 21, 233–250.
Calvo, F.J., Knapp, M., van Houten, Y.M., Hoogerbrugge, H., Belda, J.E., 2015. *Amblyseius swirskii*: What made this predatory mite such a successful biocontrol agent? Exp. Appl. Acarol. 65, 419–433.
Carrillo, D., Peña, J.E., de Moraes, G.J., 2015. Prospects for Biological Control of Plant Feeding Mites and Other Harmful Organisms. Springer International Publishing.
Claudia, H., Tom, M., Friedrich, J., 2004. Odor compounds from cyanobacterial biofilms acting as attractants and repellents for free-living nematodes. Limnol. Oceanogr. 49, 1809–1819.
Duso, C., Malagnini, V., Paganelli, A., Aldegheri, L., Bottini, M., Otto, S., 2004. Pollen availability and abundance of predatory phytoseiid mites on natural and secondary hedgerows. Biocontrol 49, 397–415.
Faryish, D.J., Axtell, R.C., 1971. Phoresy redefined and examined in *Macrocheles muscaedomestica* (Acarina: Macrochelidae). Acarologia 13, 16–29.
Ferris, H., Venette, R., Scow, K., 2004. Soil management to enhance bacterivore and fungivore nematode populations and their nitrogen mineralisation function. Appl. Soil Ecol. 25, 19–35.
Flechtmann, C.H.W., Monteiro, A.R., Lordello, L.G.E., 1980. Forésia entre ácaros e um nematoide da família Rhabditidae. Publicacao-Sociedade Brasileira de Nematologia, pp. 197–202 4.
Gerson, U., Smiley, R.L., Ochoa, R., 2003. Mites (Acari) for Pest Control. Blackwell Science, Oxford, UK.
Grosman, A.H., Messelink, G.J., Groot, E.B.D., 2011. Combined use of a mulch layer and the soil-dwelling predatory mite *Macrocheles robustulus* (Berlese) enhance the biological control of sciarids in potted plants. IOBC/WPRS Bull. 68, 51–54.
Heidemann, K., Hennies, A., Schakowske, J., Blumenberg, L., Ruess, L., Scheu, S., Maraun, M., 2014. Free-living nematodes as prey for higher trophic levels of forest soil food webs. Oikos 123, 1199–1211.
Holt, R.D., Bonsall, M.B., 2017. Apparent competition. Annu. Rev. Ecol. Evol. Syst. 48, 447–471.
Hoogerbrugge, H., van Houten, Y.M., van Baal, A.E., Bolckmans, K.J.F., 2008. Alternative food sources to enable establishment of *Amblyseius swirskii* (Athias-Henriot) on chrysanthemum without pest presence. IOBC/WPRS Bull. 32, 79–82.
Huang, H., Xu, X., Lv, J., Li, G., Wang, E., Gao, Y., 2013. Impact of proteins and saccharides on mass production of *Tyrophagus putrescentiae* (Acari: Acaridae) and its predator *Neoseiulus barkeri* (Acari: Phytoseiidae). Biocontrol Sci. Technol. 23, 1231–1244.
Kimpinski, J., Sturz, A.V., 1996. Population growth of a rhabditid nematode on plant growth promoting bacteria from potato tubers and rhizosphere soil. J. Nematol. 28, 682–686.
López, D., Vlamakis, H., Kolter, R., 2010. Biofilms. Cold Spring Harbor Perspect. Biol. 2, 1–11.
Maoz, Y., Gal, S., Argov, Y., Coll, M., Palevsky, E., 2011. Biocontrol of persea mite, *Oligonychus perseae*, with an exotic spider mite predator and an indigenous pollen feeder. Biol. Control 59, 147–157.
Messelink, G., Maanen, R.V., Holstein-Saj, R.V., Sabelis, M.W., Janssen, A., 2010. Pest species diversity enhances control of spider mites and whiteflies by a generalist phytoseiid predator. BioControl 55, 387–398.
Messelink, G.J., Bennison, J., Alomar, O., Ingegno, B.L., Tavella, L., Shipp, L., Palevsky, E., Wackers, F.L., 2014. Approaches to conserving natural enemy populations in greenhouse crops: current methods and future prospects. Biocontrol 59, 377–393.
Moreira, G.F., de Morais, M.R., Busoli, A.C., de Moraes, G.J., 2015. Life cycle of *Cosmolaelaps jaboticabalensis* (Acari: Mesostigmata: Laelapidae) on Frankliniella

occidentalis (Thysanoptera: Thripidae) and two factitious food sources. Exp. Appl. Acarol. 65, 219–226.
Munoz Cardenas, K.A., 2017. What lies beneath? Linking litter and Canopy Food Webs to Protect Ornamental Crops. Department of Population Biology, Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Amsterdam, The Netherlands, pp. 144.
Neher, D.A., 2010. Ecology of plant and free-living nematodes in natural and agricultural soil. Annu. Rev. Phytopathol. 48, 371–394.
Nomikou, M., Janssen, A., Schraag, R., Sabelis, M.W., 2002. Phytoseiid predators suppress populations of *Bemisia tabaci* on cucumber plants with alternative food. Exp. Appl. Acarol. 27, 57–68.
Nomikou, M., Sabelis, M.W., Janssen, A., 2010. Pollen subsidies promote whitefly control through the numerical response of predatory mites. Biocontrol 55, 253–260.
O'Connor, B.M., 2009. Cohort Astigmatina. In: Krantz, G.W., Walter, D.E. (Eds.), A manual of acarology. Texas Tech University Press, Lubbock, Texas, pp. 565–657.
Palevsky, E., Argov, Y., Ben David, T., Gerson, U., 2003. Identification and evaluation of potential predators of citrus rust mite. Syst. Appl. Acarol. 8, 39–48.
Pijnakker, J., Arijs, Y., de Souza, A., Cellier, M., Wäckers, F., 2016. The use of *Typha angustifolia* (cattail) pollen to establish the predatory mites *Amblyseius swirskii*, *Iphiseius degenerans*, *Euseius ovalis* and *Euseius gallicus* in glasshouse crops. IOBC WPRS Bull. 120, 47–54.
Rahman, L., Whitelaw-Weckert, M.A., Orchard, B., 2014. Impact of organic soil amendments, including poultry-litter biochar, on nematodes in a Riverina, New South Wales, vineyard. Soil Res. 52, 604–619.
Rinker, D., Bloom, J., 1982. Phoresy between a mushroom-infesting fly and two free-living nematodes associated with mushroom culture. J. Nematol. 14, 599.
Rodrigueiro, T.S.C., Prado, A.P.D., 2004. *Macrocheles muscaedomesticae* (Acari, Macrochelidae) and a species of *Uroseius* (Acari, Polyaspididae) phoretic on *Musca domestica* (Diptera, Muscidae): effects on dispersal and colonization of poultry manure. Iheringia – Série Zoologia 94, 181–185.
Rodriguez, J., Wade, C.F., Wells, C.N., 1962. Nematodes as a natural food for *Macrocheles muscaedomesticae* (Acarina: Macrochelidae), a predator of the housefly egg. Ann. Entomol. Soc. Am. 55, 507–511.
Rueda-Ramírez, D., Rios-Malaver, D., Varela-Ramírez, A., De Moraes, G.J., 2018. Colombian population of the mite *Gaeolaelaps aculeifer* as a predator of the thrips *Frankliniella occidentalis* and the possible use of an astigmatid mite as its factitious prey. Syst. Appl. Acarol. 23, 2359–2373.
Rueda-Ramírez, D., Rios-Malaver, D., Varela-Ramírez, A., de Moraes, G.J., 2019. Biology and predation capacity of *Parasitus bituberosus* (Acari: Mesostigmata: Parasitidae) on *Frankliniella occidentalis* (Thysanoptera: Thripidae), and free-living nematodes as its complementary prey. Pest Manag. Sci.
Sampson, C., 1998. The commercial development of an *Amblyseius cucumeris* controlled release method for the control of *Frankliniella occidentalis* in protected crops. Brighton Crop Protection Conference: Pests & Diseases, Brighton, UK, pp. 409–416.
Shipp, J.L., Wang, K., 2003. Evaluation of *Amblyseius cucumeris* (Acari: Phytoseiidae) and *Orius insidiosus* (Hemiptera: Anthocoridae) for control of *Frankliniella occidentalis* (Thysanoptera: Thripidae) on greenhouse tomatoes. Biol. Control 28, 271–281.
Smith, D., Papacek, D.F., 1991. Studies of the predatory mite *Amblyseius victoriensis* (Acarina: Phytoseiidae) in citrus orchards in south-east Queensland: control of *Tegolophus australis* and *Phyllocoptruta oleivora* (Acarina: Eriophyidae), effect of pesticides, alternative host plants and augmentative release. Exp. Appl. Acarol. 12, 195–217.
Smolentseva, O., Gusarov, I., Gautier, L., Shamovsky, I., DeFrancesco, A.S., Losick, R., Nudler, E., 2017. Mechanism of biofilm-mediated stress resistance and lifespan extension in *C. elegans*. Sci. Rep. 7, 7137.
Tachi, F., Osakabe, M., 2012. Vulnerability and behavioral response to ultraviolet radiation in the components of a foliar mite prey–predator system. Naturwissenschaften 99, 1031–1038.
Thoden, T., Korthals, G., Termorshuizen, A., 2011. Organic amendments and their influences on plant-parasitic and free-living nematodes: a promising method for nematode management? Nematology 13, 133–153.
Walter, D.E., Hunt, H.W., Elliott, E.T., 1987. The influence of prey type on the development and reproduction of some predatory soil mites. Pedobiologia 30, 479–1424.
Warburg, S., Inbar, M., Gal, S., Salomon, M., Palevsky, E., Sadeh, A., 2019. The effects of a windborne pollen-provisioning cover crop on the phytoseiid community in citrus orchards in Israel. Pest Manag. Sci. 75, 405–412.
Weintraub, P.G., Kleitman, S., Alchanatis, V., Palevsky, E., 2007. Factors affecting the distribution of a predatory mite on greenhouse sweet pepper. Exp. Appl. Acarol. 42, 23–35.