



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)

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ABSTRACT

The stable fly (*Stomoxys calcitrans*) and horn fly (*Haematobia irritans*) are mainly parasites of cattle that spend the early phase of their life cycle in decaying vegetation, manure and soil, as members of a wide range of other harmful and beneficial arthropods. Predators of the family Macrochelidae are considered beneficial mites that have been considered promising biological control agents of fly eggs and larvae and of other harmful organisms. The objectives of this study were: a) to compare the predation and oviposition rates of three *Macrocheles* species, *M. embersoni*, *M. muscaedomesticae* and *M. robustulus*, on six prey species: *S. calcitrans*, *Musca domestica*, *H. irritans*, *Bradysia matogrossensis*, *Rhizoglyphus echinopus* and the free living nematode *Rhabditella axei*; b) to evaluate the life cycle of the best performing predator (*M. embersoni*) on the most suitable prey species at $30 \pm 2^\circ\text{C}$, $95 \pm 5\%$ RH and in the dark. The three macrochelid species consumed all evaluated prey species, but *M. embersoni* had higher predation and daily oviposition rates on larvae of *S. calcitrans* (23.8 larvae consumed and laid about 4.0 eggs, respectively) than other species. Total immature development of *M. embersoni* was completed in at most about 1.3 days on eggs of *S. calcitrans* and *M. domestica* and 1.5 days on *R. axei*. *Macrocheles embersoni* produced most eggs (0.28–0.34 female/female/day; r_m : 0.28–0.34) on those same prey. The results of this study suggest that *M. embersoni* is a promising biological control agent of *S. calcitrans* and *M. domestica*.

1. Introduction

Several fly species are known to be harmful to human beings and other animals. One of these is the stable fly, *Stomoxys calcitrans* (L.) (Diptera: Muscidae), whose adults are considered serious parasites of different animals, especially cattle, as well as humans, in different countries, including Australia, Colombia, Congo, Costa Rica, Tanzania and the United States (Fosbrooke, 1963; Herrero et al., 1989; Mora et al., 1997; Cook et al., 1999; Broce et al., 2005; Elkan et al., 2009). In Brazil, its importance has increased tremendously in recent years, especially in the central and southeastern states, in parallel with the increased field applications of vinasse, a byproduct of the alcohol and sugar industries, in sugarcane crops. The mixture of vinasse with

sugarcane fallen sugarcane leaves constitutes a substrate for the development of immature stable flies (Cançado et al., 2013; Dominghetti et al., 2015).

The house fly, *Musca domestica* L. (Diptera: Muscidae), is one of the most common nuisance insects that as adults can vector microorganisms harmful to humans and other animals, and whose immatures develop in a variety of substrates, most often in animal manure and the soil (Legner, 1995; Mariconi et al., 1999; Skovgård and Nachman, 2004; Malik et al., 2007). The horn fly, *Haematobia irritans* (L.) (Diptera: Muscidae), is also considered an important harmful organism in several countries in Europe, Americas, Asia and non-tropical Africa. Adults of this species are cattle parasites, while immatures develop in cattle droppings (Barros, 2001; Pruett et al., 2003; Almeida et al., 2010).

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Several other soil organisms can cause severe damage to cultivated plants. Examples are the fungus gnats (Diptera: Sciaridae) and the bulb mite, *Rhizoglyphus echinopus* (Fumouze and Robin) (Astigmatina: Acaridae), which damage plant parts just below the soil surface (Gerson et al., 2003).

Control of those organisms is often made difficult for the secluded microhabitats they occupy, but chemicals are often used for their control. Thus, evaluation of the potential of natural enemies for the control of those organisms is warranted. The Macrochelidae constitute an abundant and diverse group of soil predatory mites, mostly associated with decomposing organic matter where fly larvae are commonly found (Gerson et al., 2003; Lindquist et al., 2009; Azevedo et al., 2015). This large group comprises about 520 species, some of which have been evaluated for some time for their predaceous behavior on fly larvae and immatures of other invertebrates (Krantz, 1983; Azevedo et al., 2015).

One of these species, *Macrocheles robustulus* (Berlese), has been commercialized in Europe for the control of fungus gnats, thrips (Thysanoptera) and a species of *Lyprauta* fly (Diptera: Keroplatidae). However, nothing is known about the biology and potential as control agents of the vast majority of macrochelid species. Information about some species of this group indicates that some of the insufficiently known species may be useful as biological control agents of organisms which spend all or part of their lives in the soil.

The objectives of this study were: a) to compare the predation and oviposition rates of *Macrocheles embersoni* Azevedo, Berto and Castilho (Azevedo et al., 2017), *Macrocheles muscaedomesticae* (Scopoli) and *M. robustulus* on six prey species: *S. calcitrans*, *M. domestica*, *H. irritans*, the fungus gnat *Bradysia matogrossensis* (Lane) (Diptera: Sciaridae), the bulb mite *R. echinopus* and the free living nematode *Rhabditella axei* (Cobbold) (Nematoda: Rhabditidae); b) to evaluate the life cycle of the most promising predator species when fed with the prey it best performed on the preceding evaluation.

2. Materials and methods

The study was conducted between October 2014 and November 2015. Voucher specimens of the macrochelid species studied were deposited in the mite reference collection of “Departamento de Entomologia e Acarologia, Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo”, Piracicaba, State of São Paulo, Brazil. The study was conducted in incubators, at $30 \pm 2^\circ\text{C}$, $95\% \pm 5\%$ RH and in the dark. Each experimental unit consisted of a transparent plastic Petri dish (2.7 cm in diameter x 1.2 cm in height) whose bottom was covered with a layer of 0.5 cm of a mixture of gypsum and activated charcoal (9:1; Abbatiello, 1965). This layer was maintained wet by daily addition of distilled water. The open end of the unit was sealed with a piece of transparent plastic film (Magipac®), to prevent mites from escaping.

2.1. Stock colony

Specimens of *M. embersoni*, *M. muscaedomesticae* and *M. robustulus* were collected from cattle droppings respectively at Araçoiaba da Serra, Piracicaba and Cabralia Paulista, all in São Paulo State, Brazil, in February 2014. The colonies were maintained in the laboratory in plastic units (8 cm diameter and 7 cm high), whose bottom was covered with a layer of the mixture of gypsum and activated charcoal reported in the previous paragraph, which was also maintained wet by daily addition of distilled water. Each unit was filled to 70% of its capacity with vermiculite. The predatory mites were fed with a mixture of all stages of *R. axei* and with eggs and larvae of the house fly.

Colonies of *R. axei* were maintained in plastic containers with rotting pieces of pods of *Canavalia ensiformis* L. soaked in distilled water. Larvae of house flies were maintained in moist wheat bran in a plastic cage, whereas adults were maintained in a similar, separate cage and fed a mixture of milk (100 ml) with sugar (1 g). *Bradysia matogrossensis*

and *R. echinopus* were also maintained in the laboratory in cages containing respectively a moistened commercial dog food (Deli Dog Purina®) inoculated with the fungus *Rhizopus* sp. or just the same moistened dog food. Eggs and larvae of *S. calcitrans* (stable fly) were obtained from a rearing maintained at the veterinarian entomology laboratory of “Embrapa Beef Cattle”, Campo Grande, Mato Grosso do Sul State, where larvae were fed a mixture of vegetables and proteins adapted from Christmas (1970) and adults were fed cattle blood. Eggs of *H. irritans* (horn fly) were obtained from field collected adults. Due to this fact, the number of available eggs of this last fly was limited, and thus it could be tried as prey for only two of the predator species (*M. muscaedomesticae* and *M. embersoni*).

2.2. Predation and oviposition

The method adopted was based on similar previous studies of predatory mites of the families Phytoseiidae, Rhodacaridae and Laelapidae (Furtado et al., 2007; Castilho et al., 2009; Moreira et al., 2015).

Initially, the following prey species were separately transferred to each experimental unit: 50 eggs of *S. calcitrans*, 50 first instar larvae (L1) of *S. calcitrans*, 20 eggs of *M. domestica*, 20 first instar larvae (L1) of *M. domestica*, 20 eggs of *H. irritans*, 20 first instar larvae (L1) of *B. matogrossensis*, 30 nymphs of *R. echinopus*, or a surplus amount of *R. axei* (determined in preliminary tests). In the latter case, the nematodes were maintained in each unit on a small piece of jack bean pod. Soon after, a 0–2 day old gravid adult female of a predatory mite was transferred from the stock colony to a unit. Each unit constituted a replicate and 30 replicates were considered for each species of predatory mite.

The units were examined at 12 h intervals for 11 consecutive days to determine the number of prey killed and the number of eggs laid by the predator and its survivorship. The number of nematodes killed was not determined, because of the difficulty in doing so with the adopted methodology. At each examination, prey killed were replaced and eggs laid were discarded. Eggs laid on the first day were excluded from analysis because of the possible influence of previous feeding.

Predation and oviposition rates were compared statistically between predators on each prey species. For each predator average predation rates on different prey were not compared statistically, because of their different biomasses, but average oviposition rates were. Comparison was done by ANOVA, in a completely randomized design, followed comparisons of means by Tukey's test (5%), after $(\sqrt{x} + 0.5)$ transformation.

2.3. Biological parameters of the most promising predator

Based on the results obtained in the previous study, the biology of the most promising predator was evaluated on the most suitable prey species.

A life table study was initiated with fifty 0–3 h old predator eggs, each in an experimental unit. After eclosion, each predator was fed *ad libitum* with one of the selected prey. The units were examined every 3 h to determine the duration of each immature stage. After reaching adulthood, females were paired with males taken randomly from the colony. Twenty four hours later, the males were removed, to prevent cannibalism. The units were examined daily at 7 AM and 7 PM, to determine the duration of each adult phase as well as oviposition, discarding the eggs.

To determine the survivorship and possible oviposition of the predator in the absence of prey, 25 female deutonymphs (distinctly large, posteriorly rounded and yellowish, in comparison with the smaller, posteriorly tapered and whitish male deutonymphs) were each isolated in an experimental unit similar to those previously described. The experimental units were maintained without prey but moistened by daily addition of distilled water to the absorbing base of the units. After mites reached adulthood, the units were examined every 24 h, to determine survivorship.

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