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# Predation potential and biology of *Protogamasellopsis posnaniensis* Wisniewski & Hirschmann (Acari: Rhodacaridae)

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## ABSTRACT

Rhodacaridae are cosmopolitan mites mentioned as predators, although nothing is known about their potential as biological control agents. One of the objectives of the work reported in this paper was to evaluate the potential of *Protogamasellopsis posnaniensis* (Acari: Rhodacaridae) as predator of representative species of insects of the families Sciaridae (*Bradysia matogrossensis* (Lane)) and Thripidae (*Frankliniella occidentalis* (Pergande)), of mites of the family Acaridae (*Tyrophagus putrescentiae* (Schrank) and *Rhizog-lyphus echinopus* (Fumouze & Robin) and of nematodes of the family Rhabditidae (*Protorhabditis* sp.). Another objective was to determine the biological cycle of *P. posnaniensis* when fed the prey on which it performed best in the preceding predation test. The study was conducted in a laboratory where the experimental units were maintained at  $25 \pm 1$  °C,  $97 \pm 3\%$  RH and in the dark. Although the predator was able to kill all prey species considered in this study, the most favorable prey were *T. putrescentiae*, *F. occidentalis* and *Protorhabditis* sp. Survivorship of the predator in predation tests was always 98% or higher. Life table biological parameters when the predator was fed *T. putrescentiae* were:  $R_0 = 109.29$ ; T = 19.06 days;  $\lambda = 1.28$  e  $r_m = 0.32$  female/female/day. Despite preying upon larvae of *B. matogrossensis*, eggs of the former can also be killed by the latter. The results indicated that *P. posnaniensis* is a promising biological control agent, deserving additional studies on its possible use for the control of soil pests.

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Biological Contro

## 1. Introduction

A large number of edaphic organisms can cause severe damage to cultivated plants. Larvae of several species of fungus gnats (Diptera: Sciaridae) live mainly in humid organic substrates feeding on young plant tissues and fungi. Problems caused by fungus gnats of the genera Bradysia Winnertz and Lycoriella Frey have been increasingly mentioned in the literature, especially on crops grown in protected environments (White et al., 2000; Cloyd and Zaborski, 2004). Thrips (Thysanoptera) are not characterized as soil pests, but they spend their so called "pre-pupal" and "pupal" stages in the soil. The thrips Frankliniella occidentalis (Pergande) (Thripidae) is considered a serious pest, especially on protected crops (Lewis, 1997; Higgins, 1992). Mites of the family Acaridae are commonly found in humid organic substrates. In this family, species of Rhizoglyphus Claparède often attack bulbs, roots and tubers of field grown and protected crops (Diaz et al., 2000; Zhang, 2003). Different species of nematodes (Nematoda) also cause significant damage to roots of many crops (Sasser and Freckman, 1987; Weischer and Brown, 2000).

Soil pests are most often controlled by chemicals, but resistance to those products and the growing interest for consumption of products free of chemical residues have led to intensive efforts for the development of new control methods. Development of methods involving the use of biological control agents has received considerable attention (Gerson et al., 2003).

In less disturbed environments, soil mites are often numerous. Among those, the Mesostigmata are a group largely composed of species that prey on other mites and other small invertebrates; despite this behavior, these mites may also consume non-animal food items. For this reason, some Mesostigmata are considered important predators of soil pests (Inserra and Davis, 1983; Walter, 1986; Lesna et al., 2000; Ali et al., 1997; Freire et al., 2007). Mesostigmata of the family Rhodacaridae have been reported worldwide (Van Den Berg and Ryke, 1967; Price, 1973; Evans and Till, 1979; Mineiro and Moraes, 2001; Silva et al., 2004). Despite being mentioned as predators (Lee, 1970; Krantz, 1978) there is no published information on the potential of the rhodacarids, in a strict taxonomic sense (Lee, 1970), as biological control agents.

The rhodacarid *Protogamasellopsis posnaniensis* Wisniewski & Hirschmann, 1988 was described from Poland. In 2005, a population of this species was found in southern Brazil (our unpublished observation). One of the objectives of the work reported here was to evaluate the potential of this mite as a predator of representative



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species of insects of the families Sciaridae and Thripidae, mites of the family Acaridae and nematodes of the family Rhabditidae. Another objective was to determine the biological cycle of *P. posnaniensis* when fed the prey on which it performed best in preceding predation tests.

#### 2. Materials and methods

The study was conducted between October 2005 and September 2006, in the Acarology Laboratory of "Departamento de Entomologia, Fitopatologia e Zoologia Agrícola da Escola Superior de Agricultura Luiz de Queiroz" (ESALQ), "Universidade de São Paulo" (USP), in Piracicaba, State of São Paulo, where voucher specimens of the species studied were deposited. It was conducted in incubators, at  $25 \pm 1$  °C,  $95\% \pm 3\%$  RH and in the dark. In all tests, the experimental unit consisted of transparent plastic Petri dishes (2.7 cm in diameter  $\times$  1.2 cm high).

## 2.1. Stock colonies of the organisms used in the study

Specimens of *P. posnaniensis* were obtained from a stock colony initiated in 2005, about 5 months before starting this study, with mites collected from a compost used by growers of the mushroom *Agaricus blazei* (Murrill) ss. Heinemann in the State of São Paulo, Brazil. The colony was maintained in rearing units similar to those described by Freire and Moraes (2007), at 22–27 °C, 90% ± 10% relative humidity and in the absence of light. The mites were fed a mixture of all developmental stages of *Tyrophagus putrescentiae* (Schrank) (Acari: Acaridae) that was reared on a commercial dog food (Deli Dog<sup>®</sup>) (22% moisture, 19% crude protein, 5.5% crude lipid, 9% ash, 4% crude fiber, 37.6% carbohydrate, 2% calcium and 0.9% phosphorus, according to producer–Purina).

The following species were laboratory reared to be evaluated as prey: *Bradysia matogrossensis* (Lane) (Insecta: Sciaridae), *F. occidentalis, Rhizoglyphus echinopus* (Fumouze & Robin) (Acari: Acaridae) and *Protorhabditis* sp. (Nematoda: Rhabditidae). The respective prey colonies were maintained on the following substrates: dog food inoculated with the fungus *Rhizopus* sp.; pods and leaves of *Canavalia ensiformis* L. plus pollen of *Typha angustifolia* L.; dog food; and thin layers of pods of *C. ensiformes*. The mite *T. putrescentiae* was used as a control.

#### 2.2. Predation tests

The method adopted was based on similar studies conducted with predatory mites of the family Phytoseiidae (Furtado et al., 2007; Vasconcelos et al., 2008). The bottom of each experimental unit was covered with a layer of 0.5 cm of a mixture of nine parts of gypsum and one part of activated charcoal (Abbatiello, 1965); this layer was maintained humid by daily additions of distilled water. The open end of each unit was sealed with a piece of transparent plastic film (Magipac<sup>®</sup>), to prevent organisms from escaping.

Initially, the following prey species were transferred to each experimental unit: 10 larvae of *B. matogrossensis*, 10 pupae of *F. occidentalis*, 30 nymphs of *R. echinopus*, 30 adults of *T. putrescentiae* or a surplus amount of *Protorhabditis* sp. (determined in preliminary tests). In the latter case, the nematodes were transferred and remained in each unit on a slice of Jack bean pod. Immediately afterward a gravid adult female of *P. posnaniensis* taken from the stock colony was transferred to each unit. Fifty units with each type of prey were used in this test.

The units were examined daily for 10 consecutive days to determine the number of prey killed, 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 day, prey killed were replaced and eggs laid were discarded.

Because of an apparent attack on the eggs of *P. posnaniensis* by *B. matogrossensis*, two additional tests were conducted to confirm this possibility using the same procedure previously described. Each test had 30 replicates. In the first test, each experimental unit contained a gravid adult female of *P. posnaniensis* and either 10 live larvae, 10 live larvae on a portion (about 1 g) of its feeding substrate, previously described in this paper, or 10 larvae recently killed by exposure to steam for 30 s. In the second test, each experimental unit contained 10 eggs of *P. posnaniensis* and 3 larvae of *B. matogrossensis*. In both cases, the number of eggs laid by the predator was evaluated daily for 10 consecutive days.

Data of the comparative tests were analyzed by ANOVA, in a completely randomized design, comparing the means by Tukeýs (5%) test, after  $\sqrt{x + 0.5}$  transformation.

## 2.3. Life table

This study was initiated with eggs of *P. posnaniensis* of known average age. To obtain them, 10 adult females were transferred from the stock colony to each of 6 experimental units, each containing a surplus amount of all developmental stages of *T. putrescentiae* as food. Twelve hours later, each of the eggs laid was isolated in an experimental unit. The study was initiated with 40 eggs; after eclosion, the predator was fed *ad libitum* on the same prey. The units were examined twice a day (7 AM and 7 PM) to determine the duration of each adult phase as well as oviposition. Eggs laid daily by all females were grouped in a new experimental unit, where the predators were kept up to adulthood, to determine the sex ratio. The life table parameters (Southwood, 1978) were calculated by the method proposed by Maia et al. (2000).

## 3. Results and discussion

#### 3.1. Predation tests

The daily average of each prey species killed by *P. posnaniensis* is presented in Table 1. The averages were not compared statistically because of the different biomasses of the organisms tested as prey. Concurrently, *P. posnaniensis* oviposited when fed any of the prey, but the number of eggs recovered was significantly higher when fed on *T. putrescentiae* (*F* = 594.46; *df* = 4, 245; *p* < 0.0001), and was progressively lower when fed on *Protorhabditis* sp., *F. occidentalis, R. echinopus* and *B. matogrossensis*. Survivorship rates of the predator were  $\geq$  98% regardless of the prey.

The relatively high numbers of eggs of *P. posnaniensis* recovered when fed on *T. putrescentiae*, *Protorhabditis* sp. and *F. occidentalis*, suggest that this predator should be considered for further studies on its possible use as control agent of species of those prey groups.

#### Table 1

Prey consumption, oviposition and survivorship of *Protogamasellopsis posnaniensis* on different prey species at  $25 \pm 1$  °C,  $97 \pm 3\%$  RH and in the dark.

Prey	Prey killed/ predator/day (±SE)	Eggs/predator/ day (±SE)	Survivorship <sup>*</sup> (%)
Bradysia matogrossensis	1.8 ± 0.1	0.5 ± 0.1e	98
Frankliniella occidentalis	4.3 ± 0.2	5.6 ± 0.3c	98
Rhizoglyphus echinopus	$12.8 \pm 0.4$	1.9 ± 0.2d	98
Tyrophagus putrescentiae	23.5 ± 0.7	7.6 ± 0.5a	100
Protorhabditis sp.	-	6.3 ± 0.3b	98

\* At the end of the observation period (10 days).

The high rate on *T. putrescentiae* is compatible with the easiness with which the colony of *P. posnaniensis* was initially established using that species as prey. It is possible that the predators used in this study were conditioned to feeding on this prey while maintained in the stock colony. The conditioning of an animal to its regular food has been demonstrated for other arthropods (Peacock et al., 2003).

Oviposition and prey consumption rates of *P. posnaniensis* on pupae of *F. occidentalis* were higher than those determined by Berndt et al. (2004) on the same prey for two mite species of the family Laelapidae, *Stratiolaelaps* (= *Hypoaspis*) *miles* (Berlese) and *Gaeolaelaps* (= *Hypoaspis*) *aculeifer* (Canestrini). Those laelapids have been commercially produced for the control of thrips and fungus gnats. The rate of comsumption of *R. echinopus* by *P. posnaniensis* was higher than reported by Ragusa and Zedan (1988) for *G. aculeifer* on the same prey. Despite the low determined rate of oviposition of the predator, the rate of consumption of *B. matogrossensis* was higher than determined by Wright and Chambers (1994) for *S. miles* on *Bradysia paupera* Tuomikoski. On several occasions, larvae of *B. matogrossensis* were observed to be apparently attacking eggs of *P. posnaniensis*. These observations led us to conduct two additional tests to clarify this possibility.

In the first of the additional tests, the highest the number of eggs of *P. posnaniensis* recovered (F = 64.09; df = 2, 87; p < 0.0001) was observed when it was exposed to dead larvae of *B. matogrossensis* and the lowest, when it was exposed to live larvae of *B. matogrossensis* in the absence of their food (Table 2). In the latter case, the number of eggs recovered was exactly the same as obtained in the previous test. An intermediate number of eggs *P. posnaniensis* was recovered when food of *B. matogrossensis* was also available in the experimental unit, reducing its need to feed on eggs of the predator and/or turning it more difficult for *B. matogrossensis* to find the eggs of the predator. Those findings further suggested that eggs of *P. posnaniensis* were consumed by larvae of *B. matogrossensis*.

While conducting the first additional test, it was noticed that during oviposition, the predator takes the egg with her chelicerae and searches for a place to deposit it, usually in depressions on the surface of the substrate at the base of the experimental unit. In this test, most of the eggs were deposited underneath the prey food in the treatment in which the latter was present in the unit. This observation suggested an attempt of the predator to protect its offspring by placing its eggs in a location less vulnerable to attack by other organisms or to desiccation. Rates of survivorship of the adults of *P. posnaniensis* that were initially placed in the experimental units were always high ( $\geq$ 90%). Adults dying in the observation period were apparently not attacked by larvae of *B. matogrossensis*.

In the second additional test, an average consumption of  $7.8 \pm 0.4$  eggs of *P. posnaniensis* in each experimental unit by larvae of *B. matogrossensis* was observed each day. The content of an egg could be totally consumed in about 2 min. This result demonstrated the predatory capacity of *B. matogrossensis*. Thus, despite

#### Table 2

Average oviposition and survivorship of *Protogamasellopsis posnaniensis* on larvae of *Bradysia matogrossensis* offered to it in different forms, at  $25 \pm 1$  °C,  $97 \pm 3\%$  RH and in the dark.

Treatments	Eggs/predator/ day (± SE)	Survivorship <sup>*</sup> (%)
Live B. matogrossensis	0.5 ± 0.2 c	100
Live B. matogrossensis and its food substrate	1.6 ± 0.2 b	90
Dead B. matogrossensis	2.9 ± 0.4 a	100

 $^{*}$  At the end of the observation period (10 days); referring to the adults with which the test was initiated.

the fact that *P. posnaniensis* may be a good predator of *B. matogrossensis*, the outcome of the interaction of those organisms may depend heavily on the proportion at which they co-occur.

No statistically delineated test was conducted to determine the capacity of other prey species to consume eggs of *P. posnaniensis*. However, preliminary observations were conducted by placing 30 eggs of *P. posnaniensis* in each of three experimental units and then introducing to each one 30 adults of either *F. occidentalis, R. echinopus* or *T. putrescentiae*, respectively. Observations conducted in 7 consecutive days failed to indicate any sign of predation of eggs of *P. posnaniensis* by these organisms. Gerson et al. (2003) reported predation by *R. echinopus* on parasitic nematodes and by *T. putrescentiae* on insects and nematodes, but not on mites.

## 3.2. Life table

This study was conducted using *T. putrescentiae* as food because this was the prey on which *P. posnaniensis* performed best in the predation study. The larval stage was the shortest, followed by the deutonymph; both the egg and the protonymph had approximately the same duration (Table 3). The preoviposition period was very short, corresponding to about 6% of the oviposition period of 27.4 days (Table 3). The postoviposition period was very long (about 1.4 times as long as the oviposition period); thus, longevity was also very long.

Survivorship of immatures was 100%. Only about 7.5% of the adults died before starting oviposition. Fecundity was relatively high, in comparison with species of other families of Mesostigmata. Phytoseiidae are the best studied mites of this order; their fecundity is much lower (McMurtry et al., 1970; Sabelis, 1985) than determined in this study for *P. posnaniensis*.

Only females were obtained from the eggs initially separated to start the study as well as from the eggs obtained in this study. In addition, males were never observed in field collected specimens or in specimens of the stock colony. Those findings suggest that the reproduction of this predator occurs by thelytokous parthenogenesis. This type of reproduction does not seem to be the most common so for observed for the Mesostigmata (Norton et al., 1993). However, it was reported for an undetermined species of Protogamasellopsis Evans & Purvis (Walter and Kaplan, 1990) as well as for species of the closely associated Protogamasellus Karg (Walter and Ikonen, 1989; Walter and Kaplan, 1990). It is interesting to observe that those two genera, despite their high degree of morphological similarities (and their similarity in relation to reproduction) are placed in different families. The latter is placed in the family Ascidae (Halliday et al., 1998). Thelytoky has also been reported for Rhodacarellus silesiacus Willmann and Multidentorhodacarus (= Rhodacarus) denticulatus (Berlese) (Walter and Ikonen, 1989; Walter and Oliver, 1989; Walter and Kaplan, 1990).

The biological parameters determined in this study indicated that the population of *P. posnaniensis* increased about 109 times (net reproduction rate,  $R_o$  = 109.29) every 19 days (mean generation time, *T* = 19.06), corresponding to a daily population growth of about 28% (finite rate of increase,  $\lambda$  = 1.28), that is, to the production of 0.32 female per female per day (intrinsic rate of population increase,  $r_m$  = 0.32).

Tyrophagus putrescentiae as well as other species of Astigmata are rarely found in soils of the natural vegetation of the State of São Paulo (Mineiro and Moraes, 2001), although it is where the population of *P. posnaniensis* used in this study was found. The good performance of *P. posnaniensis* in this study when offered several species of prey suggests that *P. posnaniensis* is a generalist predator. Rhodacarids are known as euedaphic mites, living mostly at some distance below the soil surface (Lee, 1970; Price, 1973), where other organisms possessing softer tegument (such as nematodes, on which it was observed to feed in the present study)

#### Table 3

Development (days ± SE) [n] and reproduction of *Protogamasellopsis posnaniensis* fed *Tyrophagus putrescentiae*, at 25 ± 1 °C, 95 ± 5% RH and in the dark.

Parameter	Value
Egg	2.9 ± 0.1 [40]
Larva	1.1 ± 0.1 [40]
Protonymph	$2.9 \pm 0.1$ [40]
Deutonymph	$1.7 \pm 0.1 [40]$
Egg– Adult	8.6 ± 0.1 [40]
Viability (egg-adult) (%)	100 [40]
Preoviposition	1.7 ± 0.1 [37]
Oviposition	27.4 ± 0.8 [37]
Postoviposição	38.8 ± 2.6 [37]
Eggs/female/day	3.0 ± 0.1 [37]
Fecundity	106.4 ± 3.0 [37]
Parental sex ratio (% of ♀)	100 [40]
Progeny sex ratio (% of ♀)	100 [3,938]

are dominant. In addition, similarly to several other groups of Mesostigmata, it may also feed on different types of fungi. Studies to determine the actual types of food items that this mite uses in the field are warranted. In addition, the promising results of this study warrants further investigation on the possibility of using *P*. *posnaniensis* or other species of the same family for practical control of soil pests. Mass production of these mites may be feasible and at relatively low cost, suggesting the possibility of releasing it periodically for pest control.

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