

Predatory Potential of *Phytoseiulus macropilis* (Banks) Preying on *Tetranychus urticae* Koch (Acari: Phytoseiidae, Tetranychidae)

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Abstract

The two-spotted spider mite, *Tetranychus urticae* Koch (Tetranychidae), is considered one of the most important species of pest-mites because it is cosmopolite and polyphagous. This species has been described as attacking over 1,100 plant species in 140 families of economic importance. On the other hand, *Phytoseiulus macropilis* (Banks) (Phytoseiidae) is a predatory mite of group I, specialist as predatory mite from the *Tetranychus* genus. Thus, the objective of this work was to evaluate predatory potential of *P. macropilis* in its different stages—nymphs, female and male adults—preying on *T. urticae* also in different stages—eggs, larvae, nymphs, and adults—and to know the functional and numerical responses in lab studies. Both the experiments were carried out on arenas made of Jack-bean leaflets' discs [*Canavalia ensiformis* (L.)—Fabaceae] with 3 cm in diameter over agar-water at 3% inside 5 cm in diameter uncapped Petri dishes. To know the predatory activity, forty *T. urticae* and one predatory mite were placed in each arena with the respective phases of the developmental life cycle to be evaluated. To know the potential of predation, the no killed mites were counted after 24 hours. To know the functional and numerical responses, immature *T. urticae* in densities of 1 to 300/arena were offered for *P. macropilis*. The results for the predatory potential showed that larvae and male adult of *T. urticae* were the most killed stages, and the female predatory mites were the one that consumed most prey. The functional response showed a positive and significant correlation, suggesting a type II functional response (convex), a cyrtoid curve rising at a decreasing rate to a plateau, where the consumption remains constant regardless

of prey density.

Keywords

Agricultural Acarology, Functional Response, Numerical Response, Biological Control

1. Introduction

The two-spotted spider mite, *Tetranychus urticae* Koch (Tetranychidae), is considered one of the most important species of mite pests because it is cosmopolite and polyphagous. This species has been described as attacking over 1,100 plant species in 140 families of economic value [1].

The mite *Phytoseiulus macropilis* (Banks) (Phytoseiidae) is a predatory mite belonging to the group I, specialist as predatory mite of mites from the *Tetranychus* genus [2]. It is known a phytoseiid tropical mite with 0.5 mm in length, presents a shape of a pear, and an orange color. This predatory mite is found in the lower surface of leaves under the two-spotted spider mite net or near the leaf mid rib. When touched, *P. macropilis* moves fast and presents an active searching behavior for the prey. A female can consume up to seven adults, 20 immatures or 25 eggs of tetranychid mites per day. Each female lays an average of 2.5 eggs/day during a period of approximately 30 days. The sex ratio is four females per male. Under field conditions, this mite occurs naturally in low populations and in many plant species in most Brazilian regions [3].

It has been observed that *P. macropilis* with *Neoseiulus californicus* (McGregor) (Phytoseiidae) are the most abundant species in strawberry cultivation (*Fragaria* spp., Rosaceae) and in plants associated with this crop, in the state of Rio Grande do Sul, Brazil [4].

Two native species of phytoseiids from Brazil, *Neoseiulus idaeus* (Denmark and Muma) and *P. macropilis*, under experimental studies for the control of the two-spotted spider mite on strawberry, were able to significantly reduce the mite population when they were released in the initial stage of infestation, presenting a great potential to be used in biological control [5] [6].

Other studies on *P. macropilis* in Brazil have been showing excellent results on the two-spotted spider mite biological control, in particular under protected growing conditions, an activity that is expanding, especially for floriculture and hydroponic vegetables, which are the crops that offer excellent conditions for the development of phytophagous mite [7] [8] [9] [10].

Based on the facts mentioned previously, the objective of the present study was to evaluate the predatory potential of *P. macropilis* on eggs, larvae, nymphs, and adults of *T. urticae* and to know the functional and numerical responses of *P. macropilis* preying on *T. urticae*, both in bioassays under laboratory conditions.

2. Material and Methods

The present study was performed under controlled conditions with temperature at $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$, $70 \pm 10\%$ of RH and 14 hours of photophase in the Acarology Laboratory of the EPAMIG Sul—Research Center of Ecology Management of Pests and Plant Diseases—EcoCentro, Lavras, Minas Gerais, Brazil. The pest mite *T. urticae* and predatory mite *P. macropilis* were both obtained from the Federal Institute of Inconfidentes, Minas Gerais, Brazil.

2.1. Lab Rearing of *Tetranychus urticae*

For rearing the pest mite, *T. urticae* were placed on upper surface of the Jack-bean leaflets [*Canavalia ensiformis* L. (DC)—Fabaceae], weekly changed [11].

Uncapped Petri dishes (15 cm in diameter) were used, with a 1 cm thick foam maintained moist with distilled water and that covered the entire bottom surface of each dish with a Jack-bean leaflet placed on top of the foam and surrounded by strips of hydrophilic cotton wool that were also in contact with the damp foam in order to avoid mites from escaping and to better conserve the leaflet.

2.2. Lab Rearing of *Phytoseiulus macropilis*

Rectangular arenas of black flexible PVC plastic sheets (26×22 cm) were used. These were put over StyrofoamTM of equal size and placed over water inside plastic trays ($32 \times 26.5 \times 5.5$ cm) [12]. Hydrophilic cotton strip was placed around the StyrofoamTM and the arena, and it was in contact with water from the tray. The cotton was used to avoid mites from escaping, in addition to preserving the Jack-bean leaflet. The leaflet was placed in arenas with the petiole under moist cotton and were infested by *T. urticae* that served as food for predatory mites, and after wilting other new leaflet, infested by *T. urticae*, was placed over the old one [13].

2.3. Predatory Potential

The experiments of *P. macropilis* predatory potential on *T. urticae* under laboratory conditions were carried out on arenas made of Jack-bean leaflet discs with 3 cm in diameter over agar-water at 3% inside 5 cm in diameter uncapped Petri dishes. The dishes were covered with PVC film to avoid mites from escaping. Predatory mites, as well as pest mites, were obtained from maintenance laboratory rearing. The predatory mites were fasted for 24 hours before they were placed on the experimental arenas.

Experiments were carried out using all possible combinations among the stages of the predatory mite development life cycle—nymphs, adult male and adult female—and each stage of development life cycle of *T. urticae*—egg, larvae, nymphs, adult male and adult female—in other words, a factorial 3×5 , with experimental design completely randomized. Forty *T. urticae* were placed on each arena in the respective stages to be studied, with 10 replications per treatment.

After 24 hours of introducing the pest mites, killed, naturally dead and lives were counted.

Larvae of *P. macropilis* were not used in the tests because this stage is completed in less than a day. In addition, according to some researchers, mites in larval stage does not eat or eats very little [14] [15] [16], and thus does not justify an investigation on *P. macropilis* in this stage.

Regarding the calculation of predatory capacity of *P. macropilis*, the data obtained were subjected to variance analysis and the means were compared with one another through the Tukey test at 5% significance, with the use of SigmaPlot software, version 9.01 [17].

2.4. Functional and Numerical Responses

Adult females of *P. macropilis* were confined for eight days on arenas of 3 cm in diameter made with Jack-bean leaflet discs inside uncapped Petri dishes of 5 cm in diameter, containing agar-water at 3%, as mentioned previously.

Pest mites, constituted only of immature stages of *T. urticae*, were placed on arena 3 cm in diameter, as already mentioned, in the following amounts: 1, 2, 5, 10, 20, 30, 35 (with seven replications), 45 (with four replications), 55 (with three replications), 70, 100, 125, 200, and 300 (with two replications). The variations in the replications occurred due to the large number of mite specimens that were necessary in the highest densities [18].

Immature stages—larvae and nymphs—of *T. urticae* were used as food for *P. macropilis* because, in general, these are the stages of the pest mite most preyed by phytoseiids [19] [20] [21]. Arenas with the same amount of *T. urticae* and without the predatory mite were maintained as a control group in order to observe the natural mortality. The numbers of dead prey as well as of the eggs laid by the predatory mite were evaluated every 24 hours along six days, when the dead prey not preyed and eggs were removed. During those six days the number of prey was replaced until it reaches the initial amount.

The functional response of the *P. macropilis* predatory mite was found through the Holling model [22]. First, a logistic regression Model (1) was fitted to determine the shape of the functional response curve of the predator/prey interaction, considering the ratio of attacked prey (N_a/N_o) as a function of the density of prey offered (N_o) [23].

The cubic logistic regression model takes on a polynomial function as a linear predictor in order to describe the linear relation between N_a/N_o and N_o and L_0 , L_1 , L_2 and L_3 as the representation of the intercept, linear, square, and cubic coefficients, respectively. The maximum likelihood method was used to estimate the coefficients. If $L_1 > 0$ and $L_2 < 0$, thus the ratio of prey consumed is supposedly a density that is positively dependent, configuring a Type III functional response. In contrast, if $L_1 < 0$, the ratio of prey offered exhibits a monotonic decline with the initial number of prey offered, configuring a Type II functional response [23].

$$\frac{N_a}{N_o} = \frac{\exp(L_0 + L_1 N_o + L_2 N_o^2 + L_3 N_o^3)}{1 + \exp(L_0 + L_1 N_o + L_2 N_o^2 + L_3 N_o^3)} \quad \text{Model (1)}$$

Once the type of functional response has been defined, a model that relates the number of prey attacked by *P. macropilis* female mites as a function of the density of *T. urticae* can be considered.

Among the models of functional response, the Holling model is illustrated by Model (2) [22]:

$$N_a = \frac{aTN_o}{1 + aN_oT_h} \quad \text{Model (2)}$$

where a is the parameter associated with the predator attack rate, T_h is the predator search time for the prey (handling time), T is the prey exposure time to the predator (in this case, it was 1 day) and N_o is the number or density of prey offered. Model (2) is non-linear in parameters and its parameters were estimated by the method of least squares [22] [23].

The numerical response was evaluated based on a square regression model, illustrated by Model (3):

$$y = b_0 + b_1 N_o + b_2 N_o^2 \quad \text{Model (3)}$$

in which b_0 , b_1 and b_2 are the model coefficients or parameters, N_o is the density of prey offered, and y is the predator mean daily oviposition. Parameters of Model (3) were estimated by the least squares method. Eventually, the weighted regression, considering the weight given by the reverse density of prey offered ($1/N_o$), was taken into account in order to stabilize the response variance in Model (3).

All statistical analyses were carried out with the assistance of the statistical software R [24].

3. Results

3.1. Predatory Potential

Nymph stages, female and male adults of *P. macropilis* are able to preying on all stages of *T. urticae*. Larvae and adult males of *T. urticae* were the most preyed by all stages of the predatory mite, and the two-spotted spider mite adult female was the least preyed (Table 1, Figure 1).

Adult females of *P. macropilis* were the most efficient in the predation, preying on approximately 65% of larvae, followed by adult males that consumed about 50% of the two-spotted spider mite larvae (Figure 1).

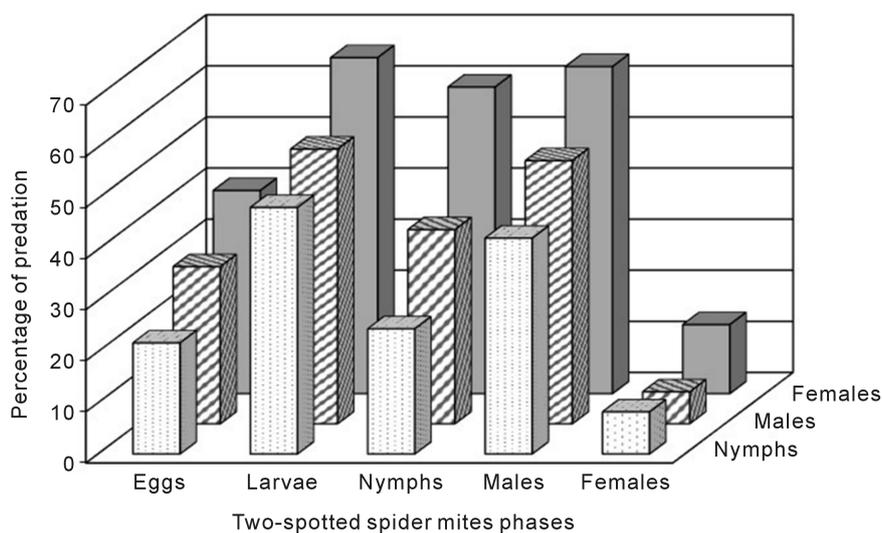
3.2. Functional Response

The estimated parameters by the logistic regression of the dead prey rate (N_d/N_o) versus the number of prey offered (N_o) to *P. macropilis* females in a day, are shown in Table 2 and these data show that the linear coefficient (L_1) was lower than zero, suggesting that the rate of prey consumed decreased in relation to the

Table 1. Number of *Tetranychus urticae* killed in its different developmental stages by nymphs and adults (male and female) of *Phytoseiulus macropilis* predatory mite (n = 40).

Stages of <i>Tetranychus urticae</i>	Mean \pm SE [*] of <i>Tetranychus urticae</i> killed by all stages of <i>Phytoseiulus macropilis</i>		
	Nymphs	Females	Males
Eggs	8.7 \pm 1.1 bB	15.9 \pm 1.7 bA	12.3 \pm 0.9 bcAB
Larvae	19.3 \pm 1.2 aB	26.3 \pm 1.2 aA	21.5 \pm 1.8 aAB
Nymphs	9.8 \pm 1.7 bB	24.0 \pm 1.5 aA	15.2 \pm 1.8 abB
Adult males	16.9 \pm 1.1 aB	25.6 \pm 1.1 aA	20.6 \pm 1.4 aB
Adult females	3.3 \pm 0.5 cAB	5.4 \pm 1.1 cA	2.5 \pm 0.4 dB

^{*}Mean \pm SE (mean standard error) followed by the same lower case letter on the columns and the same capital letter on the rows do not differ among themselves by the Tukey test ($P > 0.05$).

**Figure 1.** Percentage of all *Tetranychus urticae* developmental stages killed by nymph, male, and female of *Phytoseiulus macropilis*.**Table 2.** Parameters estimation of the cubic logistic regression model fitted to the data of *Phytoseiulus macropilis* mite attack rate on the prey *Tetranychus urticae*.

Parameter [*]	Estimation	Standard error	Value <i>P</i>
L_0	-0.1754	0.05502	0.00143
L_1	-0.01216	0.00192	<0.00001
L_2	0.00002	0.000016	0.18620
L_3	-0.00000002	0.00000003	0.44465

^{*} L_0 , L_1 , L_2 and L_3 = representation of the intercept, linear, square, and cubic coefficients, respectively.

number of prey offered, which configured a type II functional response (convex) according Holling models, a cyrtoid curve rising at a decreasing rate to a plateau, where the consumption remains constant regardless of prey density. This model presented a good fit to the data, which was indicated by the coefficient of deter-

mination value R^2 (Figure 2 and Table 3).

The curve of the functional response of *P. macropilis* preying on *T. urticae* is shown in Figure 2.

The coefficients of *P. macropilis* female mites attack rate (a) and handling time (T_h) on densities of the prey, *T. urticae*, estimated by the Holling equation [22], are represented in Table 3.

3.3. Numerical Response

The oviposition by *P. macropilis* females increased with the density of prey offered until it reached an approximate density of 162.5 *T. urticae* mites per arena 3 cm in diameter, with a maximum oviposition of 1.76 eggs/female/day, and after this density the female oviposition began to decrease (Figure 3).

The estimated parameters by the square regression model to the numerical response are represented in Table 4.

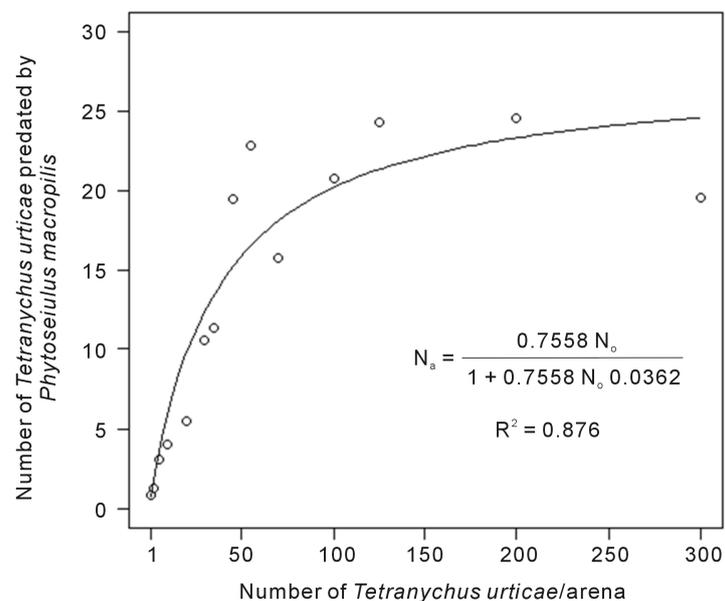


Figure 2. *Phytoseiulus macropilis* type II functional response to the amount of *Tetranychus urticae* offered, based on the Holling model [22].

Table 3. Fitted logistic model parameters estimation and coefficient of determination (R^2) regarding the data of the number of killed preys by *Phytoseiulus macropilis* female mite in different densities of the prey *Tetranychus urticae*.

Model	Parameter*	Estimation	Standard error	Value P	R^2
Holling 1959	a	0.7558	0.1935	0.0021	0.876
	T_h	0.0362	0.0042	<0.0001	-
	a/T_h	20.8710	-	-	-
	$K = 1/T_h$	27.6160	-	-	-

* a = predator attack rate, T_h = handling time (predator search time for the prey), and T = prey exposure time to the predator (in this case, it was 1 day) and, K = maximum predation rate.

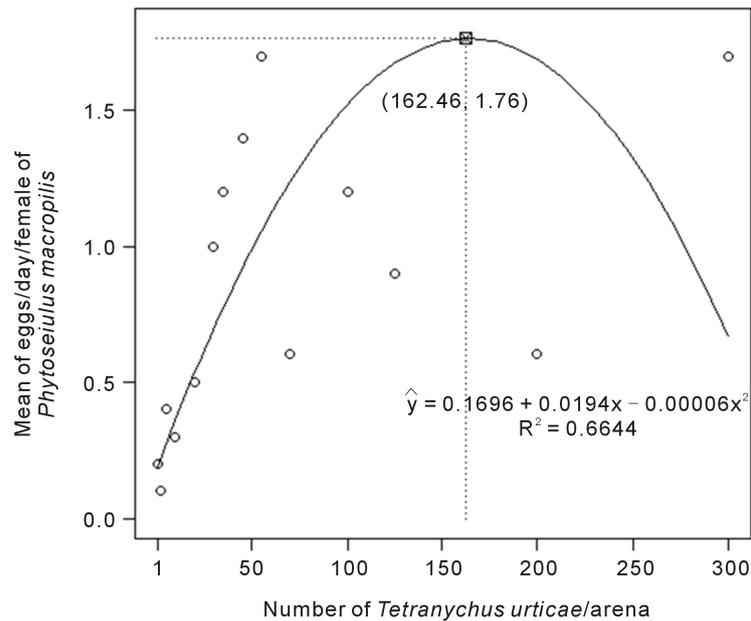


Figure 3. Numerical response of *Phytoseiulus macropilis* female to the densities of the prey, *Tetranychus urticae*, obtained by the quadratic weighted regression model.

Table 4. Parameters estimation of the quadratic weighted regression model fitted to the data of the mean number of eggs laid/day by *Phytoseiulus macropilis* female in different densities of the prey *Tetranychus urticae*.

Parameter ^a	Estimation	Standard error	Value P
L_0	0.1696	0.05395	0.0093
L_1	0.01945	0.00464	0.0015
L_2	-0.00006	0.00002	0.0234

^a L_0 , L_1 and L_2 = representation of the intercept, linear, and square, coefficients, respectively.

4. Discussion

4.1. Predatory Potential

A study of the biology of *P. macropilis* fed on *Tetranychus tumidus* Banks (Tetranychidae) showed that the nymphs and adults consumed more eggs and larvae than the other mite developmental stages; also that the female predatory mites was the one that consumed most prey, followed by males [14] and in the present study with *T. urticae* the results are similar.

In the evaluation of *P. macropilis* predatory potential in the control of two-spotted spider mite, it was observed that the female predatory mite consumed all stages of the prey; however, the eggs stage was the most consumed and the adult female prey was the least consumed [9].

A study on the management of *T. urticae* in roses (*Rosa* spp., Rosaceae) growing in greenhouse and using *Neoseiulus* sp. predatory mite has demonstrated that females consume a larger number of larvae and nymphs under controlled conditions [20].

It is also known that the predation of *T. urticae* by *N. californicus* is low on leaves that present trichome such as in the tomato plant (*Solanum lycopersicum* L., Solanaceae). This low predation was related to trichomes present on tomato leaves, one of the attributes of the plant that must be considered as an essential component for biological control practices [25]. In contrast, this phenomenon does not occur in rosebushes (*Rosa* spp.), for instance, and since trichomes are not found on rose leaves, the predatory mite is benefited once there is no physical barrier that impedes its searching capacity [20]. A study on the predation of *T. urticae* in rosebushes by *N. californicus* concluded that all predatory mite stages consumed a larger amount of the pest immature stage, and the adult females were the most efficient, followed by adult males [21].

The present study shows that as well as in other studies cited previously, the *P. macropilis* adult predatory mites (females and males) consumed a larger number of preys than nymphs stage. However, those studies, in general, report a larger consumption of eggs, larvae, and nymphs, differing from the present study, in which larvae, males, and nymphs were the most consumed. This difference perhaps can be explained by the fact that none of the studies cited above evaluated the predation on *T. urticae* males. Also, the adult females of predatory mite consume more prey than the other stages of the predatory mite perhaps due to higher energy expenditure required for the oviposition [26] and because adult females of predatory mite are much bigger than your prey *T. urticae*. Although some studies have reported low values for *P. macropilis* predation [14] [15] [16], the present study has shown that, regardless of which post-embryonic stage the pest is going through, the predatory mite is able to preying on the pest, so it can be concluded that *P. macropilis* exhibits a great potential for controlling *T. urticae* in greenhouse conditions, similar to other results found in the literature.

4.2. Functional Response

The functional response is an important aspect in population dynamics, once it is an expression of the relation between the predator's consumption and the prey's density rate [27].

The type II functional response, found in the present study for *P. macropilis*, is the most commonly reported for phytoseiid predatory mites when the density of the prey is increased. This occurs when the attack rate (a) and the handling time (T_h) do not have a relation with the prey densities [28], which was also observed in the present study.

The functional response has also been used to evaluate other parameters such as the effect of *N. californicus* long-term feeding [28], the two-spotted spider mite eggs' consumption under laboratory conditions [29], the residual toxicity of contaminated eggs with pyrethroid insecticides to females of *N. californicus* and *P. macropilis* [30], and the influence of the female and of its size in the functional response of the predator *Nephus includens* (Kirsch) (Coleoptera: Coccinellidae) [27].

The predatory mite handling time limits the response to the predatory activity. Nevertheless, an increase in the handling time (T_h) leads a reduction of the maximum predation rate (K) [27]. In the present study, the maximum predation rate was of approximately 28 *T. urticae*/day (Table 3), a similar value that was found for the phytoseiid *Neoseiulus longispinosus* (Evans) predatory mite when preying on nymphs of *Oligonychus coffeae* (Nietner) (Tetranychidae) [31].

The maximum predation estimation can be used to determine the ideal number of predatory mites that must be released in order to reduce the pest mite population. However, the predation rate under laboratory conditions might not be the same in the field situation [27].

Some researchers have studied the functional response of various predatory mites only with the prey eggs as food [28] [29] [30] [31] [32]. The present study investigated the functional response on all *T. urticae* immature stages, which is the type of predation that can be useful in the prevention of pest population increase [31].

4.3. Numerical Response

The results found in the present study, about increase of female oviposition with de increase of prey density, are similar to those found in a study on the effect of *Brevipalpus phoenicis* (Geijskes) (Acari: Tenuipalpidae) density in the functional and numerical response of *Euseius alatus* DeLeon and *Iphiseiodes zuluagai* Denmark and Muma [18]; additionally, to the results of the study on *Euseius concordis* (Chant) functional and numerical response [33]; finally to the results of the evaluation of *E. alatus* predation potential [34]. All of these mites are phytoseiids, like *P. macropilis* in this work.

The oviposition of *P. macropilis* decline when prey density is increased above 170 *T. urticae*/arena (Figure 3), and this case could have occurred due to the interference-stimulus phenomenon [35] [36]. As the prey density increases, the predatory mite suffers a great disturbance as it may spend less time in an individual prey because another prey can accidentally touch the predatory mite, making the abandon of the prey it was preying on initially to preying on another one. Therefore, the predatory mite can partially prey on the mite of the pest, instead of completely preying, which could interfere in the predator's nutrition and, consequently, in the number of laid eggs.

In a general way, another aspect that may be taken into account is the prey nutrition, which in high densities can also be impaired, since the food source (plant) is already decayed because of the intense attack due to the high density of pest mites present. Consequently, this will influence the predatory mite nutrition, especially that of the adult female [18] [31].

The oviposition correlates with the preying on because phytoseiids allocate a great fraction of the ingested food for the egg production [37]. Predatory mites survive under low prey density per leaf, but hardly lay all eggs of its potential [31]. In the present study, *P. macropilis* survived at all prey densities offered;

nevertheless, at least 50 preys were necessary so that the female to put only 1 egg/day and of at least 160 preys to reach the maximum mean oviposition of 1.76 eggs/female/day (**Figure 3**), which corresponds to a density of 23 *T. urticae*/cm².

However, under natural conditions this prey consumption and oviposition values could be different, because another factor that needs to be considered is that, in general, phytoseiids females depend on the presence of males or at least once they have been mate in order to produce eggs [38] [39]. In some cases, the females may even stop the oviposition in the absence of the male [40].

In the present study, the maximum daily oviposition of *P. macropilis* was 1.76 eggs. This value may be considered low because the females were confined in arenas during eight days in the absence of the male, which was a necessary procedure to conduct the experiment to quantify only the adult female predatory activity alone.

The increase of the predatory mite oviposition in response to the increase in the prey density can contribute in the efficacy of biological control, allowing an increase in the predatory mites' population. This is a favorable condition for *P. macropilis* because this mite, in general, is considered more efficient at high populations of the pest mites [41].

5. Conclusion

The obtained results allow concluding that all post-larval developmental stages of *P. macropilis* are efficient to kill all phases of *T. urticae* developmental cycle and with a type II functional response.

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