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NEOSEIULUS CALIFORNICUS (MCGREGOR, 1954) PREYING IN DIFFERENT LIFE STAGES OF *TETRANYCHUS URTICAE* KOCH, 1836 (ACARI: PHYTOSEIIDAE, TETRANYCHIDAE)

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ABSTRACT — The predaceous mite *Neoseiulus californicus* is one of the major biological control agents of tetranychids in greenhouses of several countries. The two-spotted spider mite *Tetranychus urticae* is one of the main pests affecting rose (*Rosa* spp.) cultures in Brazil. Chemical methods are used for its control, causing a significant environmental impact. Thus, this work aimed to study the predatory potential of *N. californicus* as an agent of biological control of *T. urticae* on roses. For the predatory capacity studies, 40 *T. urticae* mites /arenas of Jack bean leaves (*Canavalia ensiformis*) were offered to one specimen of each life stage of *N. californicus*. The adult females were the most efficient in preying upon immature stages, followed by nymphs. For the functional and numerical responses, adult females of *N. californicus* were confined to arenas made of Jack-bean leaves, and offered immature stages of *T. urticae* at the following densities: 0.14, 0.28, 0.70, 1.4, 2.8, 4.2, 4.9, 6.3, 7.7, 9.8, 14.1, 17.6 and 28.2 / cm^2. The number of killed prey (functional response) and the number of eggs laid by the predator (numerical response) were assessed every 24 hours for 8 days. A type II functional response was inferred through a regression analysis. *N. californicus* preyed on a maximum of 60 specimens of *T. urticae* per adult female per day.

KEYWORDS — Agricultural Acarology; predatory mite; two-spotted spider mite; *Rosa*; biological control

INTRODUCTION

The two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) is a major pest in greenhouses. The stability conditions of temperature and humidity maintained for plant growth also generally favour the rapid development of this pest (Zhang 2003).

The two-spotted spider mite usually causes chlorotic or discoloured spots on the leaves, which later turn into bronze and get dry. Ultimately, defoliation, sometimes total, occurs (Cruz 2000). Other damages caused to cultures are: plant growth reduction and web formation and depreciating the final product (Barbosa 2003). The production and consumption of ornamental flowers and plants in Brazil have followed the expanding world market trend (Junqueira and Peetz 2002). Roses, *Rosa*

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spp., are the major cut flower products in Minas Gerais (Landgraf and Paiva 2005). In Brazil, the rose-growing area is of 426 ha (Junqueira and Peetz 2002), with Minas Gerais accounting for 35.38 % of the production (Landgraf and Paiva 2009). Pest control is one of the challenges faced by ornamental flower and plant growers, with any foliage and flower injury caused by insects and other arthropods being unacceptable for marketing (Carvalho *et al*. 2009). In spite of often being the only strategy adopted by producers, chemical control of *T. urticae* has become increasingly difficult due to the rapid development of resistance to pesticides and decrease in the number of products registered for use (Zhang 2003; Sato *et al*. 2004, 2005). As a result, producers may increase pesticide doses, causing health risks to labour workers, environment and consumers (Kim *et al*., 2006). Predatory mites of the family Phytoseiidae are considered the most effective natural enemies for biological control of pest mites (Moraes *et al*. 2004).

The first consistent and well-documented attempts to use predatory mites as a means of controlling agricultural pests date from the second half of the twentieth century. However, pest mite control projects using predatory mites, as well as ecological studies under field conditions are still scarce in Brazil (Moraes 2002). The species *Neoseiulus californicus* (McGregor) (Phytoseiidae) (Reis *et al*. 2005) is among the main predatory mite used in greenhouses. Phytoseiid mites are the subject of many taxonomic, biological and ecological studies for being mainly predators, and of great importance in the control of phytophagous mites, especially those belonging to the family Tetranychidae (McMurtry and Croft 1997).

Assessment of the functional response and predatory capacity of Phytoseiidae when offered *T. urticae* is a critical step in determining their ability to regulate preys.

The functional response concept first described by Holling (1959) has been widely utilized to evaluate effectiveness of predacious insects and mites (Laing and Osborn 1974; Everson 1980; Sabelis 1985; Trexler *et al*. 1988; De Clercq *et al*. 2000; Badii *et al*. 2004; Reis *et al*. 2003, 2007; Timms *et al*. 2008). The functional response of the predator to the prey can follow one of three following mathematical models: Type I (linear), Type II (convex) or Type III (Sigmoid). In Type I model, the proportion of prey consumed increases linearly with prey availability up to a maximum. In Type II model, the proportion of prey consumed declines monotonically with prey density. Type III model depicts a sigmoid relationship in which the proportion of prey consumed is positively density- dependent over some ranges of prey density (Holling, 1959, 1961; Trexler *et al*. 1988; De Clercq *et al*. 2000; Timms *et al*. 2008).

Thus, the objective of this work was to study the predatory capacity, functional and numerical responses of the predatory mite *N. californicus* as a contribution to its use as an agent of biological control of *T. urticae* in rose greenhouses.

Materials and methods

Two-spotted spider mite rearing

Adults of *T. urticae* were collected from rose (*Rosa alba* L.) leaves under greenhouse conditions at the "Fazenda Experimental da Empresa da Pesquisa Agropecuária de Minas Gerais – EPAMIG", in Lavras, MG, Brazil, without pesticide application. Rearing was conducted at the Acarology Laboratory of the "Centro de Pesquisa em Manejo Ecologico de Pragas e Doenças de Plantas – EcoCentro, EPAMIG" , at the "Universidade Federal de Lavras - UFLA", Lavras, MG, at 25 $\pm$ 2 °C, 70 $\pm$ 10 % RH and 14 hours photophase, on Jack-bean [*Canavalia ensiformis* (L.) DC.] leaves placed on a disk of foam (1 cm thick) covering the bottom of a Petri dish 15 cm in diameter, without cover, and constantly moistened with distilled water. Hydrophilic cotton was placed around the leaves as a physical barrier to prevent mite escape, according to the technique of McMurtry and Scriven (1965).

Predatory mite rearing

Adults of *N. californicus* were collected from Jack-bean leaves in a greenhouse without pesticide application, at the "Instituto Federal de Educação, Ciência e Tecnologia do Sul de Minas Gerais", Inconfidentes Campus. The mites were placed on flexible black plastic PVC arenas (3 cm in diameter), floating in distilled water in Petri dishes (15 cm in diameter), without cover, according to the methodology of Reis and Alves (1997). These arenas were remained in the Acarology Laboratory, EPAMIG/ EcoCentro, at a temperature of 25 ± 2 °C, 70 ± 10 % RH and 14 hours photophase. The predatory mites were fed with castor bean (*Ricinus communis* L.) pollen and *T. urticae* from the stock rearing colony.

Studies of predator abilities

The predatory capacity potential of *N. californicus* was evaluated through bioassays in arenas (3 cm diameter), constituted of leaves of Jack-bean plant floating in distilled water in Petri dishes (15 cm diameter), without cover, according to methodology adapted from Reis *et al*. (1998).

The possible combinations between each stage of development of the predatory mite *N. californicus* [larva, nymphs (protonymph and deutonymph), adult female and male] and each phase of development of the pest-mite *T. urticae* [egg, larvae, nymphs (protonymph and deutonymph) and adult] were studied. Four bioassays, (one for each phase of development of *T. urticae*), were carried out in a completely randomized design. In each arena, for each phase of the predator, 40 pest-mites from the rearing stock were supplied in the phase intended for the study, with 10 replications.

The evaluations were made after 24 hours, counting the number of *T. urticae* consumed by the predatory mite. The mean daily mite consumed were compared with variance analyses and subsequent mean comparison test (Tukey test). The data were transformed in $\sqrt{x+0.5}$ (Ferreira 2008).

For the functional response (number of preys killed) and numerical response (number of eggs laid), adult females of *N. californicus* were confined into arenas as previously described. Immature stages of *T. urticae* were offered to one *N. californicus* adult female. Larvae and nymphs were used based on the prey's preference in immature stages for predation by phytoseiids (Gravena *et al*., 1994; Reis *et al*., 2000), at the following densities: 0.14, 0.28, 0.70, 1.4, 2.8, 4.2 and 4.9 (seven replicates), 6.3 (four replicates), 7.7 (three replicates), 9.8, 14.1, 17.6 and 28.2 / cm^2 (two replicates) according to Reis *et al*. (2003, 2007) methodology. The number of replicates decreased when *T. urticae* densities increased due to the difficulty in handling a large number of mites.

The number of preys consumed (functional response) and the number of eggs laid by the predator (oviposition rate) were evaluated every 24 hours for 8 days. These data (consumed preys and number of eggs) were submitted to regression analyses (Ferreira 2008). The number of preys was daily replaced up to the limit of the initial number in each prey density.

Results and discussion

Predatory capacity potential

All the life stages of *N. californicus* consumed more larvae of *T. urticae* than other stages. The adult females were the most efficient consuming 86.3 %, followed by the adult males with 54.8 %, the nymphs

Table 1: Number of *Tetranychus urticae* mites (mean ± SE) preyed at their different life stages by larva, nymphs (protonymph plus deutonymph), and adult (male and female) of the predatory mite *Neoseiulus californicus* (n = 40)

Treatment	Number of *Tetranychus urticae* mites by phases of development[1]							
(Predator stages)	Egg		Larva		Nymphs		Adult	
Adult (Male)	14,6 ± 0,90	b B	21,9 ± 2,93	b A	12,1 ± 2,06	b B	0,9 ± 0,23	a C
Adult (Female)	8,2 ± 0,91	a C	34,2 ± 1,54	c A	18,7 ± 2,22	c B	0,5 ± 0,22	a D
Nymphs	13,1 ± 1,98	b AB	18,7 ± 2,67	ab A	6,6 ± 0,92	ab BC	1,4 ± 0,37	a C
Larva	6,2 ± 0,64	a AB	9,9 ± 1,92	a A	3,0 ± 0,49	a BC	0,8 ± 0,29	a C

[1]Means followed by same lowercase letter in the columns and uppercase letter in the lines do not differ by Tukey's test (P ≤ 0.05)

with 46.8 % and the larvae with 24.8 % as also observed by Cedola *et al.* (2001) (Table 1 and Figure 1).

Similar results were observed by Forero *et al.* (2008) in studies with *N. californicus* in rose cultivation in Sabana de Bogotá. They also reported a higher consumption of larvae and nymphs, thus confirming the predator's preference for immature forms of the phytophagous mite.

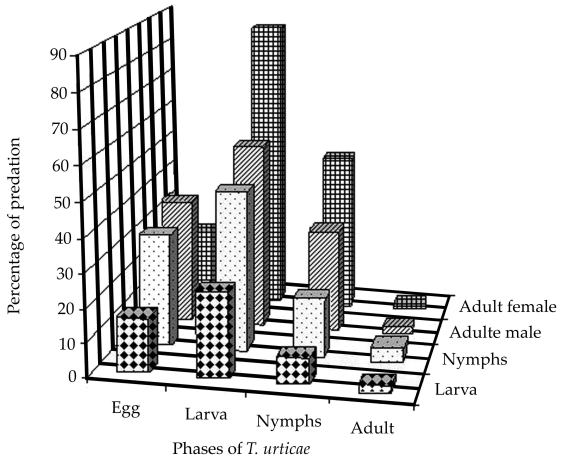

FIGURE 1: Percentage of predatory capacity of *Tetranychus urticae* at different life stages by larvae, nymphs, adult male and female of *Neoseiulus californicus*

Several studies with the predatory mite species: *Phytoseiulus persimilis* Athias-Henriot, *Galendromus occidentalis* (Nesbitt) and *N. californicus* also showed, that these predators preferred the nymphal stage than the eggs *Panonychus citri* (McGregor) in citrus, when offered simultaneously (Xiao and Fadamiro 2010).

According to Blackwood *et al*. (2001), spider mite larvae may be more profitable than eggs for generalist phytoseiids, in regards to both nutritional benefit and handling time. In their study, these latter authors observed that females of several generalist species often attempted to pierce eggs without success before attacking larvae. One possible explanation for this observation would be that generalist species might have mouthparts not as effective at piercing the *T. urticae* egg chorion as mouthparts of more specialized species. Flechtmann and McMurtry (1992) found that cheliceral characteristics differ between Phytoseiidae species primarily feeding on spider mites and pollen.

As in the present work, Cedola *et al.* (2001) evaluated the predatory capacity of *N. californicus* at the nymph stages (protonymph and deutonymph) and observed that these immature stages have a lower predatory capacity than the adult females. Such a result can be explained by the greater energy requirements for oviposition compared with the immature phases, although the larger size of the adult females also must be taken into account. All *N. californicus* phases showed a low consumption of *T. urticae* adults, compared to the other offered phases (Table 1), probably due to their greater mobility and size.

The *N. californicus* larvae were less efficient predators, likely due to their reduced size and the short duration of this stage, as also observed by Reis and Alves (1997) and Reis *et al*. (1998).

Functional Responses and Oviposition Rate

Both the functional response (preys consumed) and oviposition rate of *N. californicus* were found to increase with increasing prey density, presenting a positive and significant interaction with the coefficient of determination (r^2) of 0.94 and 0.83, respectively (Figures 2 and 3). The maximum oviposition of 1.5 eggs / day was found at the density 6.3 preys / cm^2 (Table 2).

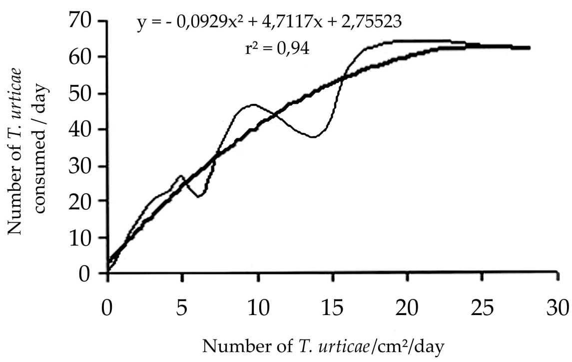

FIGURE 2: Number of *Tetranychus urticae* preyed upon by one *Neoseiulus californicus* female per day, according to the density offered (original and tendency curve)

The regression analysis results showed that the functional response is of type II (convex). The number of attacked preys by the predator's female increases asymptotically with the increasing number of offered preys. Then it gradually decelerates until reaching a level at which the predatory capacity rate becomes more or less constant, regardless of prey density.

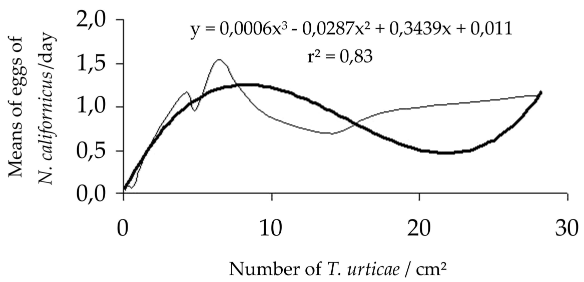

FIGURE 3: Number of eggs laid by one *Neoseiulus californicus* adult female per day, according to the density of *Tetranychus urticae* offered (original curve and trend)

Such response, according to Hassel (1978) is supposedly typical of arthropod predators. Although predators which exhibit the Type III functional response are commonly regarded as efficient biological control agents (Fernández-Arhex and Corley 2003; Pervez and Omkar 2005), many of the predators that have been successfully released as biological control agents have been shown to exhibit a Type II functional response (Xiao and Fadamiro, 2010). According to Castagoli *et al.* (2002), less clear features were evidenced for *N. californicus* that is generally considered as a specialist or selective type II predator, but it seems that an intermediate behaviour between specialist and generalist type seems more appropriate.

At a density of 14.1 preys / cm^2, the percentage of predation decreases fewer than 50 %, suggesting a predator's satiation, or that there may have been interference in its ability to prey due to increased prey density (Sandness and McMurtry 1970; Reis *et al.* 2003), indicating that *N. californicus* will be more efficient at densities between 10 and 20 preys / cm^2 than for higher ones (Table 2).

Koehler (1999) corroborate the observation that mite control by predators is very efficient, particularly at low prey densities, including the mite of the family Phytoseiidae.

Blümel *et al.* (2002) concluded that *N. californicus* is an efficient predator for the control of mites on rose and vegetable cultivations, also surviving for long periods without the presence of preys, and by feeding on pollen and other alternative food sources. A similar result was found

TABLE 2: Functional responses and oviposition rate of the predatory mite *Neoseiulus californicus*, having *Tetranychus urticae* as feed in the laboratory at 25 ± 2 °C, 70 ± 10 % RH and 14 hours photophase

Prey density		Mean		Range		Predation (%)
By arena	By cm^2	Mites preyed/day	Eggs laid/day	Prey	Eggs	
1	0,14	1	0,04	0 – 1	0 – 1	100
2	0,28	2	0,09	1 – 2	0 – 1	100
5	0,7	5	0,09	1 – 5	0 – 2	100
10	1,4	10	0,45	1 – 10	0 – 2	100
20	2,8	19,45	0,86	17 – 20	0 – 2	97,23
30	4,2	23,09	1,16	16 – 30	0 – 3	76,96
35	4,9	26,73	0,97	13 – 35	0 – 3	70,34
45	6,3	21,72	1,54	14 – 45	0 – 3	48,26
55	7,7	35,83	1,25	21 – 55	0 – 4	65,15
70	9,8	46,68	0,88	39 – 63	0 – 3	66,7
100	14,1	38,19	0,69	27 – 52	0 – 3	38,19
125	17,6	62,43	0,94	50 – 80	1 – 2	49,95
200	28,2	61,94	1,13	52 – 69	1 – 2	30,97

by Forero *et al.* (2008) with the same predatory mite preying upon *T. urticae*. Phytoseiid mites are an ecologically diverse group of species, including oligophagous specialist predators of spider mites of the genus *Tetranychus* to broadly polyphagous generalists feeding on mites, insects, fungi, plant fluids and pollen (McMurtry and Rodriguez 1987; McMurtry and Croft 1997; Pratt *et al.* 1999). Species have been classified as either specialists or generalists according to diet breadth (McMurtry and Rodriguez 1987; Schausberger and Croft 2000a, b) and according to life-style types based on diet breadth and other related biological and morphological features (McMurtry and Croft 1997).

For effective employment of *N. californicus* as a biological control agent, its predation activity must be better understood. The functional responses of *N. californicus* can differ depending upon a number of factors: strains with different nutritional histories (Castagnoli *et al.* 1999), environmental temperature (Gotoh *et al.* 2004) and the use of pesticides (Poletti *et al.* 2007). Ahn *et al.* (2010) studied the functional response of *N. californicus* on *T. urticae* in strawberries and concluded that the functional response of adult female was not influenced by non-glandular trichomes on abaxial leaves but was affected by temperature. Castagnoli *et al.* (1999) stated that it is important to know the host and the prey history of the predator strain used for successful biological control. However, this study has also shown that *N. californicus* has an excellent predatory capacity on *T. urticae* (60 pest-mites per adult female per day).

Conclusion

Although obtained in laboratory conditions, the results of this work allow to conclude that *N. californicus* can be considered an efficient predator of immature stages of *T. urticae*, at different densities of the prey. The results suggest that the testing should be done in a greenhouse for consolidation of biological control with *N. californicus* in integrated management systems.

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