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AUTHORS: Rana AKYAZI, Mete SOYSAL, Yunus Emre ALTUNÇ

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The prey-stage preferences of *Amblyseius swirskii* Athias-Henriot and *Neoseiulus californicus* (McGregor) (Mesostigmata: Phytoseiidae), between egg and nymph stages of *Tetranychus urticae* Koch (Trombidiformes: Tetranychidae)

Amblyseius swirskii Henriot ve *Neoseiulus californicus*'un (McGregor) (Mesostigmata: Phytoseiidae) *Tetranychus urticae*'nin Koch (Trombidiformes: Tetranychidae) yumurta ve nimf dönemleri arasındaki tercihi

Rana AKYAZI ^a, Mete SOYSAL^a, Yunus Emre ALTUNÇ ^a

^a Ordu University, Faculty of Agriculture, Plant Protection Department, Ordu, Turkey

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* Corresponding author:

Rana AKYAZI

✉ ranainak@hotmail.com

ABSTRACT

Tetranychus urticae Koch (Trombidiformes: Tetranychidae), two-spotted spider mite, is a pest mite species that feed on a wide range of plants around the world. *Neoseiulus californicus* (McGregor) (Mesostigmata: Phytoseiidae) is shown as a predatory mite to be a very efficient biological control agent of *T. urticae*. *Amblyseius swirskii* Athias-Henriot (Mesostigmata: Phytoseiidae) is another phytoseiid mite species which can feed and reproduce on a wide range of food sources, including *T. urticae*. Information about the prey stage preference of a predator is valuable for evaluations of the potential of predatory mites to control of pests. We therefore analyzed the prey-stage preferences of *A. swirskii* and *N. californicus* to eggs and nymphs of *T. urticae* at 15:15, 10:20, and 20:10 egg: nymph ratios under controlled conditions (25±1 °C, 65±5% RH and 16-h light: 8-h dark photoperiod). The experiment was carried out on abaxial surfaces of bean leaf discs with a diameter of 2 cm. Starved (for 24 h), 2-3 days-old gravid females of *A. swirskii* and *N. californicus* were used in the experiment. One predatory mite was placed individually on each bean leaf disc infested with 15:15, 10:20 and 20:10 ratios of *T. urticae* egg: nymph. After 24 h of feeding, the numbers of prey consumed for each prey stage were recorded. From the results, it is clear that while *N. californicus* showed no preference between egg and nymphs of *T. urticae*, *A. swirskii* preferred nymphs to eggs of *T. urticae*.

INTRODUCTION

Tetranychus urticae Koch (Trombidiformes: Tetranychidae) is known as an economically important and cosmopolitan agricultural pest around the world (Migeon and Dorkeld 2006-2018). *Neoseiulus californicus* (McGregor) (Mesostigmata: Phytoseiidae) is shown as a predatory mite

to be a very efficient biological control agent of *T. urticae*. *Amblyseius swirskii* Athias-Henriot (Mesostigmata: Phytoseiidae) is also a promising candidate for successful biocontrol of *T. urticae*. There has been many previous evidence for predation rate of *N. californicus* (Ahn et al.

2010, Armağan and Çobanoğlu 2013, Farazmand et al. 2012, Kuştutan and Çakmak 2009, Landeros et al. 2013, Xiao et al. 2013, Zheng et al. 2017) and *A. swirskii* females (Afshar and Latifi 2017, Fathipour et al. 2017, Xiao et al. 2013, Soleymani et al. 2016) on *T. urticae*. However, when the prey stages were offered separately to a predator, the preference cannot be truly considered. Because, the predator is not offered a choice between the prey stages (Blackwood et al. 2001, Peterson and Renaud 1989, Roa 1992). And, information about the prey stage preference of predatory mites is helpful for evaluations of their potential to control of pests. So, many previous researches have also focused on the prey-stage preference of different species of predator (Burnett 1971, Carrillo and Pena 2012, Croft and McMurtry 1972, Kasap 2010, Opit et al. 1997, Prasad 1967, van Maanen and Janssen 2008, Xu and Enkegaard 2010, Zarghami et al. 2014). In investigations conducted using *T. urticae* as prey, Blackwood et al. (2001) tested *N. californicus* for prey stage preferences between eggs and larvae of *T. urticae*. Xiao et al. (2013) tested the preferences of *N. californicus* and *A. swirskii* on leaf discs contained 40~60 eggs:0 nymph, 40~60 eggs:10~15 nymphs, 0 eggs:10~15 nymphs ratios of egg to nymph of *T. urticae*. Soleymani et al. (2016) studied food preferences of *A. swirskii* on eggs, protonymphs and deutonymphs of *T. urticae* with three treatments consisted eggs: protonymphs, eggs: deutonymphs and protonymphs: deutonymphs that ratios is 20:20.

The aim this study is to investigate the prey-stage preferences of *N. californicus* and *A. swirskii* at three different ratios of 15E:15N, 10E:20N and 20E:10N *T. urticae* egg (E): nymph (N) ratios under controlled conditions.

MATERIALS AND METHODS

Plant material

Phaseolus vulgaris L. cv. 'barbunia' (Fabaceae), pinto bean plant was selected to establish the *T. urticae* stock colony and served as the test substrate. The bean plants were grown in polyethylene pots (26x14 cm) which were filled with a mix of soil and vermiculite in a controlled climate room. Plants were watered manually as needed. In order to obtain plants with about 2-3-day-old primary leaves, seeds were planted every two days. Young fully expanded leaves were used to reduce the leaf disc deterioration (Akyazi and Hoy 2013, Akyazi et al. 2015).

Prey culture

The stock colony of *T. urticae* was reared in a climate room on bean plants at 25±2 °C temperature, 70-80% relative humidity and photoperiod of 16 light:8 dark (Akyazi et al. 2015). To maintain the colony, damaged plants were

replaced with new bean plants every 7 days. The newly-hatched *T. urticae* nymphs were introduced into the experimental areas at the beginning of the experiment. To obtain the same age groups of nymphs, ten *T. urticae* gravid females were placed on bean leaf discs for 24 h to ensure egg laying. After 24 h, the introduced mites were removed and the discs with eggs were held in a climate chamber until the eggs hatched and small nymphs were present.

Predator source and rearing

"Spical" and "Swirski-mite" (Koppert) biological products were used for the initial population of predatory mite. The colony was reared on waxed black paper discs (7.5x7.5 cm) placed on water-soaked cotton in plastic trays (13x13 cm) for 2-3 generations before being used for bioassays. Predators were fed daily a surplus of all life stages of *T. urticae* (Akyazi and Hoy 2013).

Starved (for 24 h), 2-3- days-old gravid females of *N. californicus* and *A. swirskii* were used in the experiment. To obtain female predators of the same age, firstly, the predatory mite eggs were placed separately on 1.5 cm-diameter leaf discs containing mixed stages of *T. urticae* and reared to adults. To ensure mating, one male of each predatory mite was then added to each leaf disc. After 24 h, predatory mites were taken from the rearing discs, then held without food for 24 h to obtain starved individuals.

Prey stage preference / experimental design

Experiments were performed on abaxial surfaces of 2 cm diameter bean leaf discs. To produce arenas with 10, 15, and 20 eggs (0-24 h old) for the desired ratios of eggs to nymphs, ten *T. urticae* gravid females were put on freshly prepared leaf disc arenas for 24 h egg laying, and later the females all were removed and the eggs laid on discs were counted. Excess eggs were removed to the desired ratios of eggs. The newly hatched (0-24 h old) nymphs which were uniform in size, color and brightness were then introduced into arenas.

Gravid adult females of *N. californicus* and *A. swirskii* which were uniform in size, color and brightness were then placed individually on each disc with 15 eggs:15 nymphs, 10 eggs:20 nymphs or 20 eggs:10 nymphs ratios of *T. urticae* (30 total prey each disc). The numbers of each prey stage consumed per predator were counted after 24 h feeding period. The female predators that did not oviposit within this period were excluded from the analysis to exclude unmated females (Blackwood et al. 2004). As a control, leaf disc arenas with the same densities of prey stages were kept without a predator to determine natural mortality. There was no mortality in control. The predators were starved for

24 h before trials. The number of replicates are shown in Table 1 and Table 2.

Statistical analysis

The prey-stage preferences (β index) were calculated using the following formula developed by Manly et al. (1972).

$$\beta = \left[\frac{\ln(N^1/Nc^1)}{\ln(N/Nc)} + 1 \right]^{-1}$$

N = the numbers of eggs provided

N^1 = the numbers of nymphs provided

Nc = the numbers of eggs consumed

Nc^1 = the numbers of nymphs consumed

The preference β index assigns preference values from 0 to 1 where 0.5 represents no preference. β index values greater than 0.5 represent the preference for nymphs while those less than 0.5 represent the preference for eggs. The β -value was calculated for each replicate and averaged to determine the mean β -value for each treatment (Blackwood et al. 2001, Xiao et al. 2013).

For the continuous data, the assumptions of ANOVA, normality control of the data and homogeneity of variance were tested by Levene's test. In case of fit, the variables were processed by one-way ANOVA. The mean results of the groups were compared in letters by Tukey's post-hoc test. In the case of the non-fitted for assumptions, the data was directly analyzed by the Kruskal-Wallis test and the results were compared in letters by Dunn post-hoc test. Minitab 17 and SPSS 24 (IBM) statistical softwares were used for analysis of dataset and the alpha level was 5% for all the steps.

RESULTS AND DISCUSSION

The preference value (β) calculated using the above formula of *N. californicus* at 15:15, 10:20, 20:10 *T. urticae* egg: nymph ratios were 0.47, 0.69 and 0.28, respectively. In addition, the mean β value was 0.48 (Table 1). Mean β value showed that *N. californicus* had no preference for either eggs or nymphs of *T. urticae*. However, there were significant differences among the mean β values at different prey-stage ratios offered ($p=0.000$). *N. californicus* exhibited positive switching, with a significantly increased preference for eggs in response to an increase of the relative abundance of eggs and with a significantly increased preference for nymphs in response to the increased relative abundance of nymphs (Table 1).

A detailed observation showed that when the eggs and nymphs of *T. urticae* were offered at 15:15, 10:20, 20:10 egg: nymph ratios, the egg: nymph predation rates by *N. californicus* were 29.26%:23.70%, 32.00%:37.75% and 43.50%:37.00%. And, mean egg: nymph predation rate was 34.92%:38.82%. There was a significant effect of the ratio of prey-stages offered to both egg ($p=0.009$) and nymph ($p=0.007$) predation rates of *N. californicus* (Table 1).

McMurtry and Croft (1997) classified originally *N. californicus* as a member of type II selective predators of *Tetranychus* spp. However, Croft et al. (1998) reported that it belongs to type III predator rather than type II. McMurtry et al. (2013) kept it in the type II group. Blackwood et al. (2001) found that type III generalist predators showed no prey-stage preference or preferred larvae to eggs. Specialist type-II did not show any prey-stage preference or preferred eggs. In our study, it is therefore not surprising that *N. californicus*, which is classified as type II predator, did not prefer the eggs or nymphs of *T. urticae*.

Table 1. Prey-stage preferences of *Neoseiulus californicus* at three different ratios of 15E:15N, 10E:20N and 20E:10N *Tetranychus urticae* egg (E): nymph (N) ratios

Egg: Nymph Ratio (E:N)	n	Beta Value (β)	Egg Predation Rate (%)	Nymph Predation Rate (%)
15 E: 15N	18	0.47 b	29.26 b	23.70 a
10 E: 20N	20	0.69 a	32.00 b	37.75 b
20 E: 10N	20	0.28 c	43.50 a	37.00 ab
Mean		0.48	34.92	38.82
P-value		0.000	0.009	0.007

Means within the columns are significantly different if small letters are different. ($p<0.05$)

n: The number of replicates considered in analysis.

These findings are in accordance with findings reported by Xiao et al. (2013). It had also no preference between egg and larvae stages of *T. urticae* according to Blackwood et al. (2004). However, Rezaie et al. (2017) found that *N. californicus* females preferred *T. urticae* protonymph to egg, *N. californicus* protonymph and deutonymphs preferred egg to protonymph. *N. californicus* protonymphs, deutonymphs, and females were also offered larvae, protonymphs, deutonymphs, adult females of *T. urticae* and *Thrips tabaci* Lindeman (Thysanoptera: Thripidae) larvae (1st instar) by Rahmani et al. (2016). Protonymphs and deutonymphs of *N. californicus* preferred *T. urticae* larvae or protonymphs to *T. tabaci* larvae. Predator females preferred *T. urticae* larvae, protonymphs or deutonymphs to *T. tabaci* larvae, and *T. tabaci* larvae to *T. urticae* females.

A. swirskii's prey stage preference was also determined (Table 2). The β values of *A. swirskii* with *T. urticae* as the host at 15:15, 10:20, 20:10 egg:nymph ratios were 0.70, 0.61 and 0.68, respectively. Additionally, the mean of β value was 0.66. There were no significant differences among the mean of β values at different prey-stage ratios offered ($p=0.063$). In conclusion, it would appear that *A. swirskii* preferred nymphs to eggs of *T. urticae*. It can also be said that *A. swirskii* maintained relatively constant feeding ratios when the ratio *T. urticae* eggs to nymphs was altered (Table 2).

Results also showed that when both eggs and nymphs were offered at 15:15, 10:20, 20:10 egg: nymph ratios, the egg: nymph predation rate by *A. swirskii* was 35.83%:63.96%, 46.49%:63.38% and 39.72%:62.78%. And, mean egg: nymph predation rate was 40.68%:63.37%. There was not a significant effect of the ratio of prey-stages offered to both egg ($p= 0.089$) and nymph ($p= 0.960$) predation rates of *A. swirskii* (Table 2).

McMurtry and Croft (1997) and Mcmurty et al. (2013) categorized *A. swirskii* as type III (generalist predators). Blackwood et al. (2001) declared that type III predators did not show any prey-stage preference or preferred larvae to eggs. In our study, the β values and mean predation rates showed that generalist predator *A. swirskii* preferred nymphs to eggs of *T. urticae*. On the contrary, Soleymani et al. (2016) indicated that *A. swirskii* consumed more eggs than nymphs of *T. urticae* at 20 eggs:20 protonymphs, 20 eggs:20 deutonymphs and 20 protonymphs:20 deutonymphs ratios. Moreover, Xiao et al. (2013) said that *A. swirskii* had no preference between egg and nymph stages of *T. urticae*. We think that these differences may arise because the *T. urticae* egg and nymphs were offered at different rates by the researchers. On the other hand, Xu and Enkegaard (2010) examined the prey preference of *A. swirskii* between chrysalis and active protonymphs, and chrysalis and active deutonymphs of *T. urticae*. They found that it did not show a preference between active and chrysalis stages of spider mites.

In summary, this paper argued that while *N. californicus* had no prey-stage preferences to either *T. urticae* eggs or nymphs, *A. swirskii* preferred nymphs to eggs at three 15:15, 10:20 and 20:10 *T. urticae* egg:nymph ratios. The functional responses of predatory mites may depend on prey type. *N. californicus* and particularly *A. swirskii* feed on the various type of food. So, future studies on the prey preferences of *N. californicus* and *A. swirskii* between *T. urticae* and other food sources should be performed.

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Table 2. Prey-stage preferences of *Amblyseius swirskii* at three different ratios of 15E:15N, 10E:20N and 20E:10N *Tetranychus urticae* egg (E): nymph (N) ratios

Egg: Nymph Ratio (E:N)	n	Beta Value (β)	Egg Predation Rate (%)	Nymph Predation Rate (%)
15 E: 15N	32	0.70 a	35.83 a	63.96 a
10 E: 20N	37	0.61 a	46.49 a	63.38 a
20 E: 10N	36	0.68 a	39.72 a	62.78 a
Mean		0.66	40.68	63.37
P- value		0.063 ^{ns}	0.089 ^{ns}	0.960 ^{ns}

^{ns} Means within columns are not significantly different ($p> 0.05$)

n: The number of replicates considered in analysis.

helped us to improve the manuscript. The part of this research was presented as poster at 8th Symposium of the European Association of Acrologists (Valencia, Spain, 11-15 July 2016) and 6th Plant Protection Congress with International Participation (5-8 September 2016, Konya, Turkey) and published as abstract in the abstract book.

ÖZET

Tetranychus urticae Koch (Trombidiformes: Tetranychidae), iki noktalı kırmızı örümcek, tüm dünyada yaygın olarak görülen ve farklı bitki türleri üzerinde beslenebilen zararlı bir akar türüdür. *Neoseiulus californicus* (McGregor) (Mesostigmata: Phytoseiidae), *T. urticae*'nin etkili bir biyolojik mücadele etmeni olarak bilinmektedir. *Amblyseius swirskii* Athias-Henriot (Mesostigmata: Phytoseiidae) ise, *T. urticae*'nin de içinde bulunduğu farklı besin kaynakları üzerinde beslenebilen ve üreyebilen, diğer bir phytoseiid akar türüdür. Predatörlerin, av-dönem tercihlerine yönelik bilgiler, onların etkinliklerinin değerlendirilebilmesi açısından oldukça önemlidir. Bu nedenle *A. swirskii* ve *N. californicus*'un *T. urticae*'nin yumurta ve nimf dönemleri arasındaki tercihi 15:15, 10:20, 20:10 yumurta: nimf oranlarında, kontrollü koşullar (25±1 °C, %65±5 RH ve 16 saat aydınlık: 8 saat karanlık aydınlanma) altında araştırılmıştır. Deneme 2 cm çapındaki yaprak disklerinin alt yüzeylerinde yürütülmüştür. Denemede aç (24 saat), 2-3 günlük *A. swirskii* ve *N. californicus* dişi bireyleri kullanılmıştır. *T. urticae* ile 15:15, 10:20 ve 20:10 yumurta: nimf oranlarında bulaşık olan yaprak disklerine birer tane predatör akar salınmıştır. 24 saatlik beslenme periyodundan sonra, avın tüketilen dönemleri sayılarak kaydedilmiştir. Sonuçlar *N. californicus*'un *T. urticae*'nin yumurta nimf dönemleri arasında herhangi bir tercih göstermediğini, ancak *A. swirskii*'nin nimf dönemini yumurtaya tercih ettiğini göstermiştir.

Anahtar kelimeler: biyolojik mücadele, predatör akar, iki noktalı kırmızı örümcek, yumurta, nimf, av dönemi, tercih

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