The prey-stage preferences of *Amblyseius swirskii* AthiasHenriot 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

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**ARTICLE INFO**

**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 phytoseid 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 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.
However, when the prey stages were used in the experiment. Nymphs were introduced into the arenas (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.

**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* 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.

**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).

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**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 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.

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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).

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

\(N\) = the numbers of eggs provided
\(N'\) = the numbers of nymphs provided
\(Nc\) = the numbers of eggs consumed
\(Nc'\) = 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. Mcmurty 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

<table>
<thead>
<tr>
<th>Egg: Nymph Ratio (E:N)</th>
<th>n</th>
<th>Beta Value (β)</th>
<th>Egg Predation Rate (%)</th>
<th>Nymph Predation Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 E: 15N</td>
<td>18</td>
<td>0.47 b</td>
<td>29.26 b</td>
<td>23.70 a</td>
</tr>
<tr>
<td>10 E: 20N</td>
<td>20</td>
<td>0.69 a</td>
<td>32.00 b</td>
<td>37.75 b</td>
</tr>
<tr>
<td>20 E: 10N</td>
<td>20</td>
<td>0.28 c</td>
<td>43.50 a</td>
<td>37.00 ab</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>0.48</td>
<td>34.92</td>
<td>38.82</td>
</tr>
</tbody>
</table>

| 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* 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 $\beta$ 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 $\beta$ value was 0.66. There were no significant differences among the mean of $\beta$ 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).

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.

**ACKNOWLEDGEMENTS**

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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

<table>
<thead>
<tr>
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<tr>
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<td>63.96 a</td>
</tr>
<tr>
<td>10 E: 20N</td>
<td>37</td>
<td>0.61 a</td>
<td>46.49 a</td>
<td>63.38 a</td>
</tr>
<tr>
<td>20 E: 10N</td>
<td>36</td>
<td>0.68 a</td>
<td>39.72 a</td>
<td>62.78 a</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>0.66</td>
<td>40.68</td>
<td>63.37</td>
</tr>
</tbody>
</table>

P-value: 0.063 **

**Means within columns are not significantly different ($p>0.05$)

n: The number of replicates considered in analysis.
ÖZET


REFERENCES


