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Research Article

Influence of Prey Egg Age on The Consumption Capacity of *Amblyseius swirskii* and *Neoseiulus californicus* (Acari: Mesostigmata: Phytoseiidae)[a]

Rana AKYAZI*, Yunus Emre ALTUNÇ, Mete SOYSAL

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

*Corresponding author: ranaakyazi@odu.edu.tr

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Abstract

The influence of egg age of *Tetranychus urticae* Koch (Prostigmata: Tetranychidae) on consumption capacity of *Amblyseius swirskii* Athias-Henriot and *Neoseiulus californicus* (McGregor) (Mesostigmata: Phytoseiidae) were evaluated under controlled conditions. To determine the effect of egg age on the predator's consumption rate, three different groups of *T. urticae* egg, 0-24, 24-48 and 48-72 hour-old, were offered as prey. All experiments were performed using unstarved and starved (24 hours) mated female adults of *N. californicus* and *A. swirskii* (2-3 day old). Each predatory mite was individually placed on each leaf disc containing 30 *T. urticae* eggs. After 24 hours of exposure, the predatory mites were removed and the number of consumed eggs was recorded for each treatment. All experiments were carried out on bean leaf discs (2 cm in diameter) at 25°C ± 1, 65 ± 5% R.H. and a photoperiod of 16 Light:8 Dark. The result showed that the consumption capacities of *N. californicus* and *A. swirskii* were influenced by eggs age of *T. urticae*. The amounts of older *T. urticae* eggs consumed by starved *N. californicus* and *A. swirskii* were higher than younger eggs'. The prey consumption was also significantly higher than when predators were deprived for prey for 24 h on a leaf. However, the effect of host egg age on the preference, fecundity and longevity of the predatory mites should also be needed further studies.

Key words: Predatory mite, starvation, consumption, egg age, twospotted spider mite, biological control.

Amblyseius swirskii ve *Neoseiulus californicus* (Mesostigmata: Phytoseiidae)'un Tüketim Kapasitesine Avın Yumurta Yaşının Etkisi

Özet

Amblyseius swirskii Athias-Henriot ve *Neoseiulus californicus* (McGregor) (Mesostigmata: Phytoseiidae)'un tüketim kapasitesine, *Tetranychus urticae* Koch (Prostigmata: Tetranychidae)' nin yumurta yaşının etkisi, kontrollü koşullar altında araştırılmıştır. Bu amaçla, av olarak *T. urticae*'nin 0-24, 24-48 ve 48-72 saatlik olmak üzere 3 farklı yaştaki yumurtaları kullanılmıştır. Deneyler, *N. californicus* ve *A. swirskii* (2-3 günkük)' nin deneme öncesi 24 saat açlığa maruz bırakılmış ve tok olan döllenmiş ergin dişi bireyleri kullanılarak yürütülmüştür. Her predatör akar, üzerinde 30 adet *T. urticae* yumurtası bulunan fasulye yaprak diskleri üzerine, bireysel olarak yerleştirilmişlerdir. 24 saat sonra predatör akarlar diskler üzerinden uzaklaştırılarak, tüketilen yumurta sayıları kaydedilmiştir. Deneyler 2 cm çaplı fasulye yaprak diskleri kullanılarak, 25°C ± 1, 65 ± 5% nisbi nem ve 16A:8K (Aydınlık: Karanlık) aydınlanma koşullarında yürütülmüştür. Sonuçlar, *N. californicus* ve *A. swirskii*'nin tüketim kapasitelerinin *T. urticae*'nin yumurta yaşından etkilendiğini göstermiştir. Açlığa maruz bırakılmış *N. californicus* ve *A. swirskii* bireylerinin, yaşlı *T. urticae* yumurtalarını, genç olanlara göre daha fazla tükettikleri de belirlenmiştir. Ayrıca, açlığa maruz bırakılan predatör akarların tüketim miktarlarının önemli derecede arttığı da tespit edilmiştir. Fakat ileriki çalışmalarda, yumurta yaşının predatör akarların besin tercihi, üreme kapasitesi ve ömürleri üzerine etkileri de araştırılmalıdır.

Anahtar kelimeler: Predatör akar, açlık, tüketim, yumurta yaşı, iki noktalı kırmızı örümcek, biyolojik mücadele.

Introduction

Tetranychus urticae Koch (Prostigmata: Tetranychidae) is one of the most important pests that feed on plants around the world. *Neoseiulus californicus* (McGregor) (Mesostigmata: Phytoseiidae) is widely used as a biological control agent for *T. urticae*. *Amblyseius swirskii* Athias-Henriot (Mesostigmata: Phytoseiidae) is also a promising agent for the effective *T. urticae* control.

Consumption capacity of predators is important for their effectiveness. The predators require energy to reproduce and survive. If the preys contain deficient nutrients, the predator will starve and unable to produce enough offspring (Helle and Sabelis, 1985). Eggs at different ages may have different nutrient content. So, consumption capacity of predatory mites may change when they feed on *T. urticae* eggs at different ages. The following studies were conducted on the consumption capacities of the two mentioned phytoseiid species on the same or mixed *T. urticae* eggs of different ages. For instance, Xiao et al. (2013) determined that the maximum daily predation by *A. swirskii* and *N. californicus* were 15.1 and 25.6 *T. urticae* eggs day^{-1}, respectively. Armağan and Çobanoğlu (2013) studied consumption capacity of *N. californicus* on *T. urticae* and indicated that predatory mite's daily consumption capacity was 16.33 eggs. But, these consumption capacities can be affected by prey egg age. For example, Cavalcante et al. (2015) examined potential consumption of *A. swirskii* on different age (0-24 and >48 hours) of *Bemisia tabaci* (Hemiptera: Aleyrodidae) eggs. And, they observed higher predation rates for eggs up to 24 h of age in comparison with older eggs.

In particular no study, to our knowledge, has considered the influence of *T. urticae* egg age on the consumption capacity of any predatory mite species at different levels of hunger. We therefore analyzed the consumption capacity of 24 h-starved and unstarved *N. californicus* and *A. swirskii* females on *T. urticae* eggs of different ages and investigated whether egg age and hunger affected the predation rate of the two mentioned phytoseiid species.

Material and Methods

Plant and mite rearing

Phaseoulus vulgaris L. (Fabaceae), Pinto bean plants, were used as a host for *T. urticae.* Plants were reared in a mixture of vermiculite and soil in plastic pots (26 x 14cm) in a climate room (Akyazi and Hoy, 2013; Akyazi et al., 2015).

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).

Spical and Swirski-Mite (Koppert) commercial biopreparats were used for initial population of *N. californicus* and *A. swirskii*, respectively. The colony was reared on waxed black paper discs (7.5x2.5 cm) placed on water soaked cotton in plastic tray (13x13 cm) for 2-3 generations before being used for bioassays. The predatory mites were feed mixed life stages of *T. urticae* daily (Akyazi and Hoy, 2013).

Influence of prey egg age on the consumption capacity of Amblyseius swirskii and Neoseiulus californicus

Experiments were performed using bean leaf discs (2 cm in diameter). To produce arenas with 30 prey eggs, 10 adult females of *T. urticae* were introduced on bean leaf discs for oviposition and kept overnight. After 24 hours, the introduced mites were removed. The eggs laid on leaf discs were counted. Excess eggs were removed to equalized 30 eggs. 0-24 hours old eggs were used for experiment immediately. The remaining eggs were maintained to the desired eggs old for 24-48 and 48-72 hours. Since the eggs began to hatch after 72 hours, the consumption capacity of predator mites for *T. urticae* eggs older than 72 hours could not be investigated.

To study the influence of prey egg age on the consumption capacity, two groups of predatory mites were used; one starved for 24 h and one not starved gravid females of *N. californicus* and *A. swirskii* (2-3 days old). For this purpose, firstly, the predatory mite eggs were placed on 1.5 cm-diameter leaf discs containing mixed stages of *T. urticae*, separately 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 and then held without food for 24 h to obtain starved individuals. And to calculate unstarved consumption rate, predators were not removed to the rearing discs for 24 h. Starved and unstarved females were then placed individually onto each leaf disc with 0-24, 24-48 and 48-72 hours old eggs of *T. urticae*, separately. The numbers of each eggs consumed were counted after 24 h of feeding. The number of replicates for each treatment can be found in Figure 1-2 and ranged from 30 to 47.

Statistical analysis

To analysis the influence of prey egg age on the consumption capacity of *N. californicus*, the Kolmogorov-Simirnov and Levene's tests were used to test normality and homogeneity of variance, respectively. The Datas were analyzed with Kruskal - Wallis test, Mann - Whitney U test and Student t test. Means were compared with Bonferroni (Dunn) test. When significant differences were found at P<0.05, differences among samples were considered as statistically significant. The statistical analysis was conducted with IBM SPSS Statistics 22 package program.

To analysis the influence of prey egg age on the consumption capacity of *A. swirskii*, the Kolmogorov-Simirnov and Levene's tests were applied to test normality and homogeneity of variance, respectively. The Datas were analyzed with One Way Anova test, Mann - Whitney U test and Student t test. Means were compared with Tukey's multiple comparison test. When significant differences were found at P<0.05, differences among samples were considered as statistically significant. The statistical analysis was conducted with IBM SPSS Statistics 22 package program.

Results and Discussion

In this study, the effect of prey egg age on the consumption capacities of 24 h-starved and unstarved *N. californicus* and *A. swirskii* were determined (Figure 1-2).

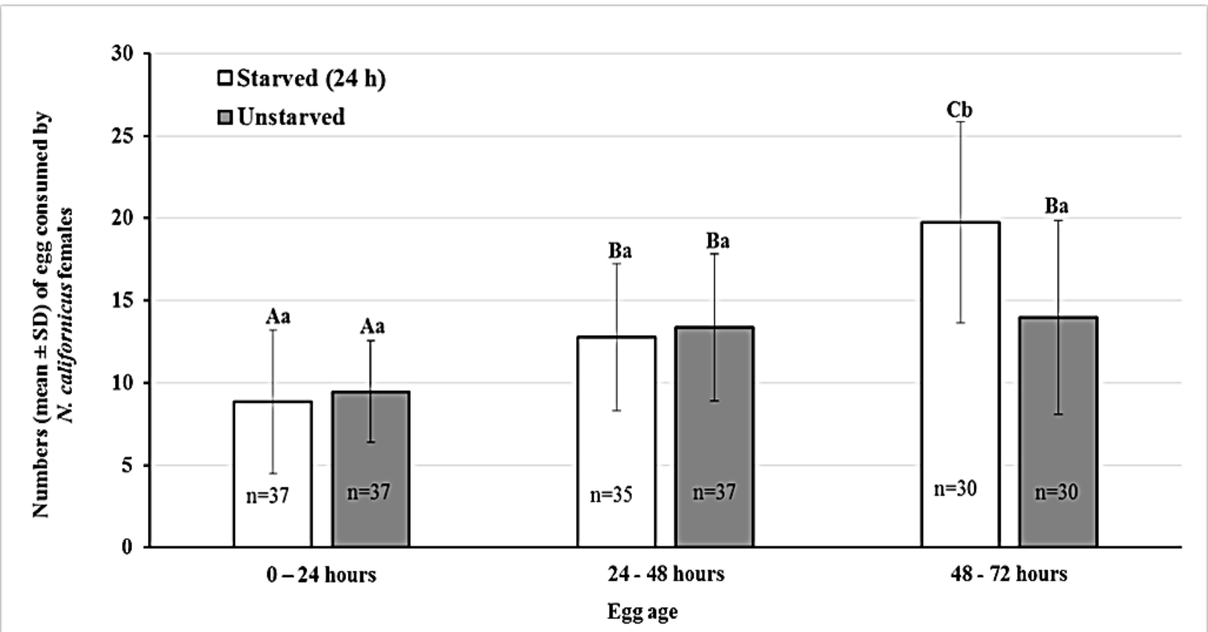

Figure 1. The consumption capacity of starved and unstarved *N. californicus* females on eggs of different age of *T. urticae*. Different upper letters represent statistically differences among the number of eggs of different age consumed at the same hunger level and different lower letters represent statistically significant differences among the starved and unstarved females for same-aged eggs (P< 0.01) (SD: Standard Deviation; n: Number of predatory mites evaluated).

The statistical analyses showed that there was significant difference between the consumption capacities of starved *N. californicus* on 0-24, 24-48 and 48-72 hours old *T. urticae* eggs (P< 0.01). The consumption of starved predators on 48-72 hours old prey eggs were higher than on the others (Figure 1).

On the other hand, there was no significant difference between the consumption capacity of unstarved *N. californicus* on 24-48 and 48-72 hours old eggs (P>0.05). The average numbers of 0-24, 24-48 and 48-72 hours old eggs consumed by starved *N. californicus* were 8.84, 12.77 and 19.77, respectively. These numbers for unstarved *N. californicus* were 9.46, 13.38 and 13.97 eggs, respectively (Figure 1).

As far as we know, no previous research has investigated the influence of *T. urticae* egg age on the consumption capacity of any phytoseiid predatory mite species. While there has been many previous evidence for predation rate of *N. californicus* females to *same* age eggs of *T. urticae*, no study has focused on the egg age of the prey. For instance, the following studies were conducted on the consumption capacity of *N. californicus* for the same age eggs of *T. urticae*. Kuştutan and Çakmak (2009) found that the number of *T. cinnabarinus* eggs (age unspecified) consumed by 1 gravid *N. californicus* female (age unspecified; 16 h starved) per day was 16.65 ± 0.62 when the predator was offered 30 eggs of *T. urticae* on bean leaf discs (3-cm diameter). Ahn et al. (2010) investigated the functional response of adult female *N. californicus* (5- to 7-day old, starved for 6 h) on *T. urticae* eggs (0-24 h old) on strawberry leaves. The number of *T. urticae* eggs consumed by *N. californicus* over a 24-

h period was 14.5 at 25 °C. It was detected by Farazmand et al. (2012) that mated adult females of *N. californicus* (1 day old, starved for 6 h) consumed 11.3±0.33 eggs within 24 h when 32 *T. urticae* eggs (age unspecified) on a cucumber leaf disc (3-cm diameter) were offered. Landeros et al. (2013) found that *N. californicus* consumed 14.67±2.52 eggs when 32 eggs of *T. urticae* (0-24 h old) per apple leaf were adjusted to one mated female predator (starvation unspecified; two days post-maturation) during a period of 24 hours at 27 ± 2ºC and 60% relative humidity. *N. californicus* (starvation and age unspecified)'s daily consumption capacity was detected as 16.33 eggs of *T. urticae* (age unspecified) by Armağan and Çobanoğlu (2013). Xiao et al. (2013) determined that the maximum daily predation by a female *A. swirskii* (starvation and age unspecified) was 15.1 *T. urticae* eggs (age unspecified) day^{-1}. Zheng et al. (2017) found that daily consumptions by female adults of *N. californicus* (< 24 h old, starved for 24 h) at different densities of eggs of *T. urticae* (0-24 h old) on bean leaf discs (3.5- cm diameter) were ranged between 8-12 eggs.

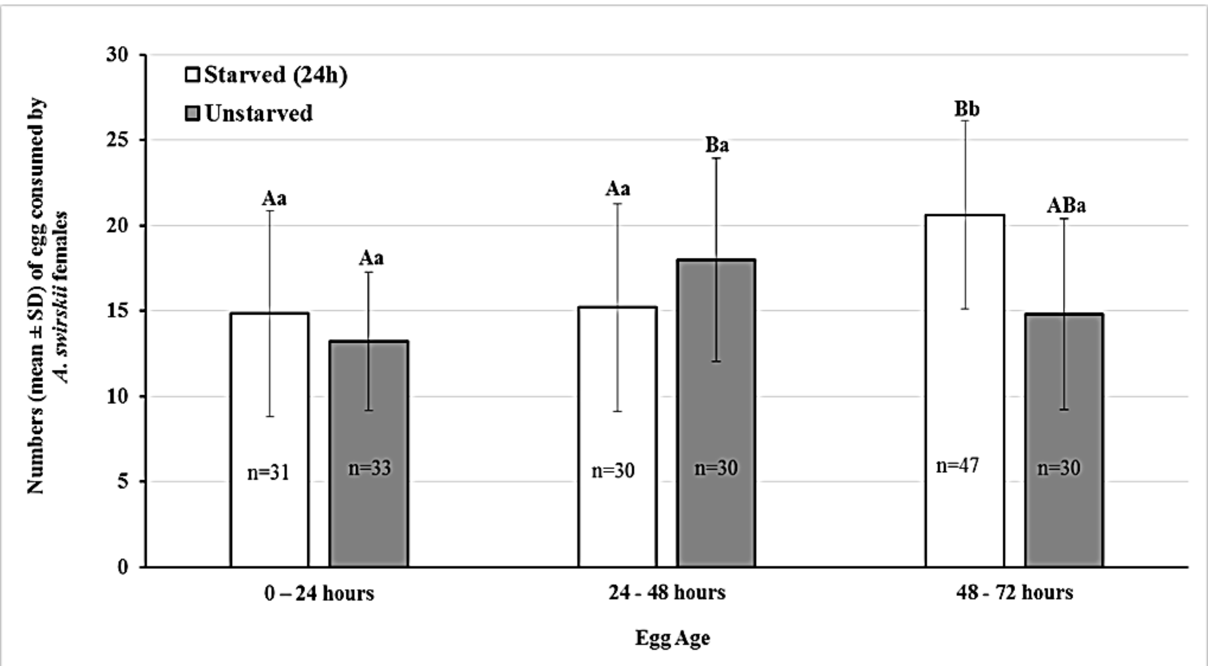

Figure 2. The consumption capacity of 24 h-starved and unstarved *A. swirskii* females on eggs of different age of *T. urticae.* Different upper letters represent statistically differences among the number of eggs of different age consumed at the same hunger level and different lower letters represent statistically significant differences among the starved and unstarved females for same-aged eggs (P< 0.01) (SD: Standard Deviation; n: Number of predatory mites evaluated).

According to the results, there was no significant difference between the consumption capacities of starved *A. swirskii* on 0-24 and 24-48 hours old prey eggs (P>0.05). But, the consumption capacities of starved predators on 48-72 hours old host eggs were higher than on the others (P<0.01). Also, statistically differences was determined between the consumption capacity of unstarved *A. swirskii* on 0-24 and 24-48 hours old eggs (P<0.01). The average numbers of 0-24, 24-48 and 48-72 hours old host eggs consumed by starved *A. swirskii* were 14.84, 15.20 and 20.62 eggs, respectively. These numbers for unstarved *A. swirskii* were 13.21, 18.00 and 14.83 eggs, respectively (Figure 2).

In particular no study, to our knowledge, has also considered the influence of prey egg age on the consumption capacity of *A. swirskii*. Most early studies as well as current work focus on the predation rate of *A. swirskii* females to same or mixed age eggs of *T. urticae* as prey. For instance, Xiao et al. (2013) determined that the maximum daily predation by a female *A. swirskii* (starvation and age unspecified) was 15.1 *T. urticae* eggs (age unspecified) day^{-1}. When 30 *T. urticae* eggs (age unspecified) were offered to adult females of *A. swirskii* (2 days old, starved for 24 h.) by Soleymani et al. (2016), in 24 h, the predator consumed 17 ± 0.74 *T. urticae* eggs on bean leaves. Afshar and Latifi (2017) found that daily predation rate of 24 h starved, 3-day old *Amblyseius swirskii* females to 24 h-old eggs of *T. urticae* on strawberry was 25.72 ± 0.12 egg day^{-1}. The predation rate of *A. swirskii* on eggs of *T. urticae* was determined under laboratory conditions using cucumber leaf discs by Fathipour et al. (2017). When, 32 *T. urticae* eggs (24 h-old) were offered to 3-day-old, unstarved *A. swirskii* females, daily consumption of *T. urticae* eggs by *A. swirskii* was 2.1±0.46 eggs. Cavalcante et al. (2015) determined that daily predation rate of a commercial population of *A. swirskii* obtained from Koppert (Netherland) was 25.1 *T. urticae* eggs when 0-6 h old eggs of *T. urticae* (30 eggs per bean leaf) were offered to one gravid female predator during a period of 24 hours.

Overall, no previous research has investigated the influence of egg age of *T. urticae* on consumption capacity of *A. swirskii* and *N. californicus*. Only Cavalcante et al. (2015) demonstrated that the oviposition rates of *A. swirskii* fed with 0-24 and 24-48 h old *B. tabaci* eggs were significantly higher on younger eggs in the non-choice test. Briefly, higher predation rates were observed for eggs up to 24 h of age in comparison with older eggs. Contrary to the findings of Cavalcante et al. (2015), we recorded higher predation rate when predators (especially starved) fed on older prey eggs. The factor leading to that greater predation was not evaluated. However, the older eggs may have a higher nutritional value for these predatory mites and thus result in higher predation rates.

When compared to the predation rates of starved and unstarved *N. californicus* and *A. swirskii* that feed on same-aged eggs, it is concluded that, statistically differences only occurred on 48-72 hours old eggs ($P<0.01$) (Figure 1-2). The starved predatory mites consumed more 48-72 hours old eggs than unstarved mites. As a result, the prey consumption rates of both of these phytoseiid mites upon eggs of *T. urticae* (48- 72 h old) were affected by the hunger level. A similar conclusion was reached by Sandness and McMurtry (1970). They found that predatory mites *Phytoseius floridanus* Muma, *Euseius concordis* (Chant) and *Amblyseius largoensis* (Muma) (Mesostigmata: Phytoseiidae) were affected by the hunger or satiation level. A significantly higher predation was recorded when the predator had deprived of prey. Similar conclusions were reached on different predator groups by Lovei et al. (1985), Torres et al. (2002) and Maselou et al. (2015). Contrary to the findings of the researchers mentioned above, Mori and Chant (1966) found that the differences in consumption of prey between the three levels of hunger were not significant.

Overall, our results demonstrate two things. First, the amounts of older *T. urticae* eggs consumed by starved *N. californicus* and *A. swirskii* were higher than younger eggs. Second, the consumption rates of two mentioned phytoseiid species were affected by the hunger level. And, the starved predatory mites consumed more 48-72 hours old eggs than the satiated ones. The prey consumption was significantly higher than when predators were deprived for prey for 24 h on a leaf.

We think that the prey eggs of different ages may be diets of different quality for predatory mites. Either younger or older eggs may be more nutritious for these predatory mites. It is known that if the prey is nutritionally deficient, the fecundity and longevity of predatory mites and the developmental success of their progeny are negatively affected (Helle and Sabelis, 1985). The predatory mites may also show a different preference between *T. urticae* eggs of different ages. For all these reasons, the effect of prey egg age on the preference, fecundity and longevity of the predatory mites should also be needed further studies.

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