



Predatory mite *Amblyseius orientalis* prefers egg stage and low density of *Carpoglyphus lactis* prey

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Abstract

Amblyseius orientalis (Ehara) (Acari: Phytoseiidae) is an effective predatory mite for spider mite control on fruit trees in China. In recent decades, it has been produced massively at a commercial natural enemy producer, feeding on the storage mite *Carpoglyphus lactis* (L.). In the predator production process, the ratio of predatory mites to their prey was found to be critical for the population increase of *A. orientalis* in large-scale rearings. In this study, we investigated the predatory capacity of *A. orientalis* on various developmental stages of the prey *C. lactis*, and the effect of prey numbers on predator reproduction. The maximum predation rate of *A. orientalis* adults on *C. lactis* adults was 2.21 per day at the lowest density of five prey adults, and on *C. lactis* eggs it was 45.07 at the highest density of 60 prey eggs. The preference index C_i of *A. orientalis* on *C. lactis* eggs and adults was 0.4312 and -0.9249 , respectively, suggesting that *A. orientalis* preferred eggs to adults. *Amblyseius orientalis* could reproduce when it preyed on either eggs or deutonymphs of *C. lactis*. However, the fecundity of the predatory mites is not always proportional to the provided prey number. Higher density of prey deutonymphs resulted in lower fecundity, whereas more prey eggs resulted in higher fecundity of *A. orientalis*. Therefore, our study indicated that the choice of suitable density and developmental stage of prey can significantly improve *A. orientalis* production on a large scale.

Keywords Alternative prey · Predatory capacity · Prey preference · Reproduction

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Introduction

Amblyseius orientalis (Ehara) (Acari: Phytoseiidae) is a widely distributed and effective predatory mite for spider mite control in fruit production in China (Sheng et al. 2014; Zheng et al. 2008). Previously, it was used exclusively as a spider mite control agent (Zhang et al. 1992; Zheng et al. 2008); however, in the past decade, it was found to be effective against thrips and whiteflies as well (Sheng et al. 2014; Yang et al. 2018). Thus, the mass rearing and large-scale production of this native predatory mite is gradually attracting attention. Early studies of *A. orientalis* rearing involved host plants and spider mites. However, it was not practical for large-scale production of *A. orientalis* in a natural enemy factory, as it was a long and costly process (Zhang et al. 2015). Some studies have attempted to rear predatory mites on pollen, but the cost was too high and results were not satisfactory (Liao and Zhu 1985).

Carpoglyphus lactis (L.) is a fast-reproducing storage pest mite which has been widely used as an alternative prey (Knapp et al. 2018; Ramachandran et al. 2021; Wang et al. 2008; Wu et al. 2009). It can be used for successful large-scale rearing of many commercial predatory mites, such as *Amblyseius swirskii* (Athias-Henriot) (Nguyen et al. 2013; Zhang and Zhang 2021). Our laboratory has reported that *A. orientalis* feeding on *C. lactis* can complete development, mating, and reproduction (Sheng et al. 2014). Compared with diets of *Bemisia tabaci* (Zhang et al. 2015), *Panonychus citri* (Zhang 1990) and castor pollen (Liao and Zhu 1985), *A. orientalis* feeding on *C. lactis* had a shorter preoviposition period and higher reproduction rate (Sheng et al. 2014). Due to diet compatibility, *C. lactis* appears a suitable alternative diet for large-scale production and field applications of *A. orientalis*.

Instead of spider mites, few studies have investigated whether there is a preference of *A. orientalis* to developmental stages of prey *C. lactis* as well as their predatory capacity on *C. lactis*. Moreover, we notice that the density of prey to *A. orientalis* is influential to predator population increase during both laboratory observation and factory production (personal observation). Although mass rearing method was considered with no negative impact on predation and development of the predatory mite (Saemi et al. 2017), many studies have already revealed the critical effect of prey on predation and female fecundity of the predators (Werling et al. 2013; Osman et al. 2016; Rasmy and Abou-Ellella 2002). If prey numbers were much higher than predator numbers, they could inhibit the expansion of *A. orientalis* populations instead (personal observation).

In this study, we investigated the predatory capacity of *A. orientalis* on different developmental stages of the prey *C. lactis*, the stage preference and the effect of prey numbers on predator reproduction. The aim of this study was to uncover the potential reason of low population growth of *A. orientalis* when using *C. lactis* as an alternative prey in large-scale production.

Materials and methods

Mite rearing

Amblyseius orientalis was collected from soybean fields in Changli Research Institute of Pomology, Hebei Academy of Agricultural and Forestry Sciences (119°09'E, 39°43'N). The colony had been maintained on *C. lactis* for 10 years in the Laboratory of Predatory Mites, Institute of Plant Protection, Chinese Academy of Agricultural Sciences

(Yan et al. 2022). *Carpoglyphus lactis* feeding on yeast were provided by Shoubonong Biotechnology Company (Beijing, China). All mites were reared in an incubator (RXZ, Ningbo, China) at 25 ± 1 °C, $70 \pm 5\%$ RH and L14:D10 photoperiod.

The predatory capacity of *Amblyseius orientalis*

To investigate the predatory capacity of *A. orientalis* on *C. lactis* at different densities, we transferred *C. lactis* adults and deutonymphs into small arenas (Yan et al. 2022). Briefly, the arena was a tiny device with two tightly clipped layers (modified Munger cage): a transparent acrylic board ($30 \times 20 \times 3$ mm) with a 1 cm diameter hole in the middle sealed by nylon mesh with glue, one piece of rectangular glass ($30 \times 20 \times 1$ mm) on top. The prey densities were set at 5, 10 and 20 adults or deutonymphs per arena. *Amblyseius orientalis* female adults were starved for 24 h in a plastic box before being transferred separately to each arena with *C. lactis* adults or deutonymphs. All treatments were repeated with 30 *A. orientalis* adults. The consumed number of both stages of *C. lactis* was recorded within 24 h.

Similarly, we investigated the predatory capacity of *A. orientalis* adults and larvae on eggs of *C. lactis* at different densities. We collected sufficient *C. lactis* eggs to set three densities of 30, 45 and 60 per arena. *Amblyseius orientalis* females were starved for 24 h and then separated into the arena with different densities of prey eggs. The same process was repeated, with one newly hatched *A. orientalis* larva per arena, and three prey densities of *C. lactis* were set at 10, 20 and 30 eggs per arena.

Stage preference of *Amblyseius orientalis* to prey

We studied the developmental prey stage preference when *A. orientalis* was exposed to egg, nymph, and adult in the same arena. We collected one starved *A. orientalis* female adult and transferred it to the arena with 10 eggs, 10 deutonymphs and 10 adults of *C. lactis*. The predation number of *A. orientalis* on each developmental stage of *C. lactis* was recorded after 6, 12 and 24 h. The experiment was replicated 30×.

Effect of developmental stage and density of *Carpoglyphus lactis* on predator fecundity

We moved one gravid *A. orientalis* adult into the arena with densities of 30, 45 and 60 eggs or 5, 10 and 20 deutonymphs of *C. lactis*. Each density was repeated 30×. These arenas were changed daily with the same number of eggs or deutonymphs of *C. lactis*, respectively. We then recorded the number of consumed eggs or deutonymphs of *C. lactis* daily and the oviposition of each *A. orientalis* for 7 consecutive days.

Statistical analysis

Kruskal-Wallis tests with pairwise comparisons were used to examine the effects of different *C. lactis* densities on predatory capacity, prey preference and fecundity of *A.*

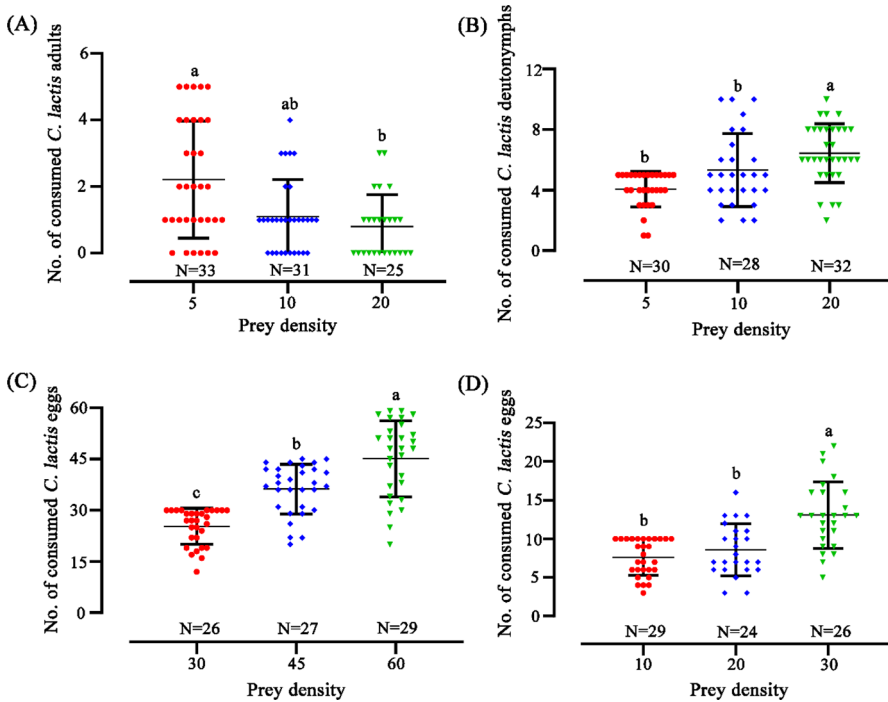


Fig. 1 Predatory capacity (number of prey items consumed) of *Amblyseius orientalis* on *Carpoglyphus lactis* at different densities. Predation of **A** *A. orientalis* adults on *C. lactis* adults, **B** *A. orientalis* adults on *C. lactis* deutonymphs, **C** *A. orientalis* adults on *C. lactis* eggs, and **D** *A. orientalis* larvae on *C. lactis* eggs. Each dot represents an individual. Horizontal lines indicate the mean (\pm SD) of biological replicates. Means capped with different letters are significantly different (Kruskal–Wallis test; $p < 0.05$)

orientalis ($\alpha = 0.05$). All analyses were run in SPSS v.25.0 and results were visualized in GraphPad v.8.02.

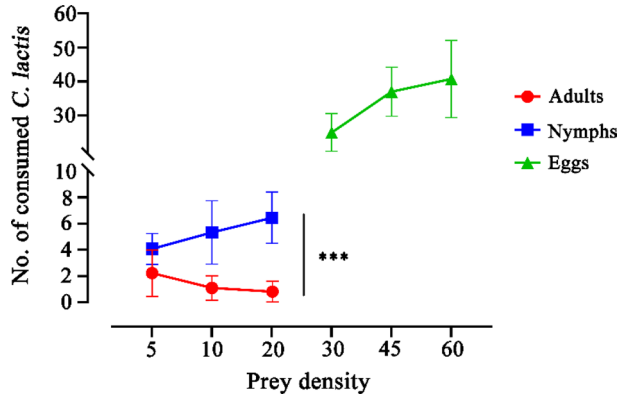
In the preference experiment, the prey preference index (C_i) of predators to various stages was expressed as $C_i = (Q_i - F_i) / (Q_i + F_i)$ (Ivlev et al. 1962; Zhou and Chen 1987), where F_i is the proportion of the i th prey type in the environment, and Q_i is the proportion of predation of the i th prey type by the predator. If $C_i = 0$, the predator had no preference for the i th prey. If $0 < C_i < 1$, the predator had a positive preference for the i th prey, and if $-1 < C_i < 0$, the predator had a negative preference for the i th prey.

Results

The predatory capacity of *Amblyseius orientalis* on *Carpoglyphus lactis*

Prey densities significantly affected predation but in different trends (Fig. 1). The maximum number of *C. lactis* adults preyed upon by *A. orientalis* females was 2.21 at the lowest density of five mites per arena ($\chi^2 = 12.436$, $df = 2$, $p = 0.002$; Fig. 1A). In contrast, only 0.80 killed prey was observed at the density of 20 *C. lactis* adults per arena. The opposite tendency was observed when *A. orientalis* fed on deutonymphs: at the

Fig. 2 Predatory capacity (mean \pm SD) no. prey items consumed) of *Amblyseius orientalis* adults on various life stages of *Carpoglyphus lactis* at different densities. Asterisks indicated significant differences between densities (Kruskal–Wallis test: $p < 0.05$)



highest density of 20 mites per arena the maximum predation number was 6.44, which was significantly more than 4.07 in the group of five prey mites per arena ($\chi^2 = 22.414$, $df = 2$, $p < 0.001$; Fig. 1B). When testing *A. orientalis* adults and larvae on *C. lactis* eggs, the consumption number increased with prey densities. The maximum predation number of *A. orientalis* adults was 45.07 at the highest density of 60 eggs per arena, in contrast to the predation of 25.30 on 30 eggs per arena ($\chi^2 = 44.869$, $df = 2$, $p < 0.001$; Fig. 1C). Similarly, the maximum predation number of *A. orientalis* larvae was 13.08 at the highest density of 30 eggs per arena, which was more than the 7.62 in an arena with 10 prey eggs ($\chi^2 = 24.536$, $df = 2$, $p < 0.001$; Fig. 1D). In total, taking *C. lactis* eggs and deutonymphs as preys, the predation of *A. orientalis* adults tended to increase with prey density, whereas the predation number tended to decrease when feeding on high density of *C. lactis* adults ($\chi^2 = 26.336$, $df = 1$, $p < 0.001$; Fig. 2).

Stage preference

Amblyseius orientalis significantly preferred eggs to deutonymphs and adults of *C. lactis* (Table 1). Of the *C. lactis* eggs 81.8% was consumed, the largest percentage. The positive index C_i of 0.431 indicated a preference of *A. orientalis* for *C. lactis* eggs. On the contrary, *C. lactis* adults were the least chosen with only 1.3%. The negative index C_i of -0.925 suggested that *A. orientalis* have a strong aversion towards adult prey.

In the 24 h preference experiment, the numbers of consumed *C. lactis* eggs in the three time periods were 3.87, 5.87, and 8.43, which were significantly higher than the respective adult numbers of 0, 0.04, and 0.09 (Fig. 3). Apparently, *A. orientalis* preying on eggs of *C. lactis* continued at each time period.

Effect of prey developmental stage and density on *Amblyseius orientalis* fecundity

The above experiment revealed different preferences of *A. orientalis* to eggs and deutonymphs of *C. lactis*, so we further tested the effects of eggs and deutonymphs at different densities on *A. orientalis* fecundity. The maximum predation was 4.15 killed prey at the highest density of 20 deutonymphs per day ($\chi^2 = 8.107$, $df = 2$, $p = 0.017$; Fig. 4A). However, the highest reproduction with 2.13 eggs per day occurred at the density of five prey deutonymphs ($\chi^2 = 21.487$, $df = 2$, $p < 0.001$; Fig. 4C). When preyed on eggs, 38.62 eggs were consumed at the highest density of 60 eggs per arena, significantly more than 31.50

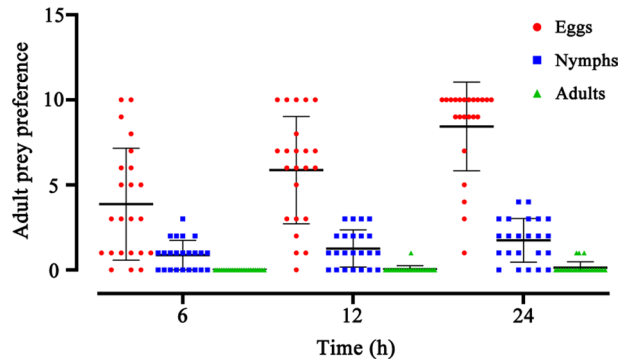
Table 1 Consumption by *Amblyseius orientalis* adults of three *Carpoglyphus lactis* life stages

Life stages	Density offered	Number consumed (mean \pm SD)	% consumed	C_i
Eggs	10	8.43 \pm 0.54a	81.8	0.4212
Deutonymphs	10	1.74 \pm 0.27b	16.9	-0.3272
Adults	10	0.13 \pm 0.072c	1.3	-0.9249

Means followed by different letters are significantly different (Kruskal–Wallis test; $p < 0.01$)

C_i : prey preference index (see "Materials and methods" Section for explanation)

Fig. 3 Developmental stage preference (no. prey items killed) of *Amblyseius orientalis* adult females after 6, 12 and 24 h, when offered 10 eggs, 10 deutonymphs and 10 adults of *Carpoglyphus lactis* simultaneously. Each dot represents an individual. Horizontal lines indicate the mean (\pm SD) of replicates



and 26.94 eggs at the other two density groups ($\chi^2 = 32.833$, $df = 2$, $p < 0.001$; Fig. 4B). The corresponding maximum reproduction was 1.90 eggs at the density of 45 prey items ($\chi^2 = 14.869$, $df = 2$, $p = 0.001$; Fig. 4D).

Predation rate and reproduction of *A. orientalis* were only correlated when prey density was high (Fig. 5). When prey number was lower, although not correlated, it did not decrease reproduction.

Discussion

The results of our study showed that *A. orientalis* female adults had strong predatory capacities on *C. lactis* eggs and deutonymphs, but not on adults. In the preference experiment, the C_i of 0.42 to *C. lactis* eggs showed a preference, whereas the C_i of -0.92 to adults indicated aversion. This is consistent with what we observed during practical rearing. Some studies also revealed predatory mites preferred eggs as prey (Furuichi et al. 2005; Ganjisaffar and Perring 2015; Moghadasi et al. 2013; Naem et al. 2017; Song et al. 1995). Aversion of adult prey by the predators might be based on semiochemical compounds – an interesting study showed that *Suidasia medanensis* after attack could release defense volatiles that deter subsequent attack from the predatory mite *A. swirskii* (Midthassel et al. 2016). Whether such chemical compounds are also involved in the interaction of *C. lactis* and *A. orientalis*, or whether perhaps a physical barrier is in play in the developmental stage preference, remains to be elucidated.

Body size and mobility affects *A. orientalis* predation. In the experiment, the starved *A. orientalis* can prey on *C. lactis* adults and deutonymphs, both of similar size as the

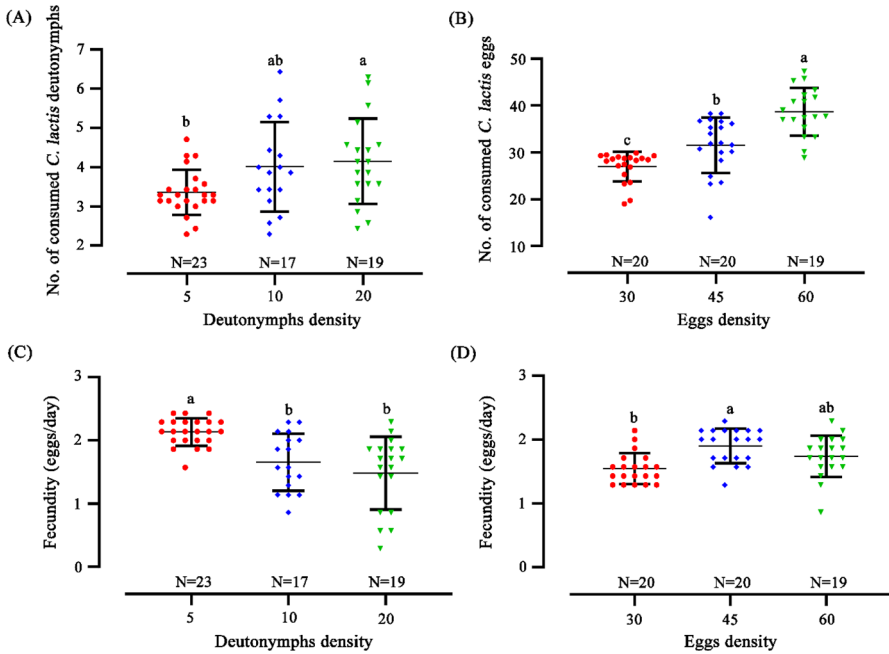


Fig. 4 Predatory capacity (no. prey items consumed) and fecundity (no. eggs laid) of *Amblyseius orientalis* adult females on eggs and deutonymphs of *Carpoglyphus lactis* offered at three densities. Predation on *C. lactis* **A** deutonymphs and **B** eggs. Fecundity on *C. lactis* **C** deutonymphs and **D** eggs. Each dot represents an individual. Horizontal lines indicate the mean (\pm SD) of biological replicates. Means capped with different letters are significantly different (Kruskal–Wallis test: $p < 0.05$)

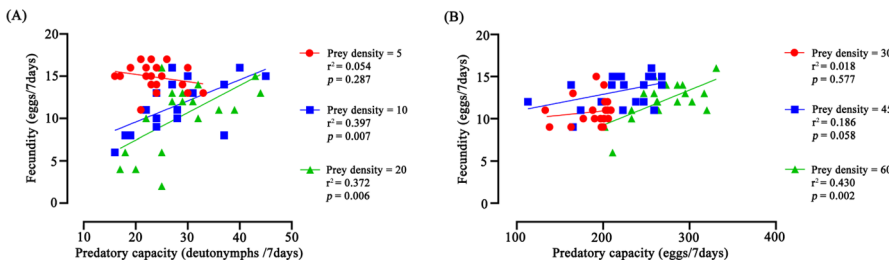


Fig. 5 Linear relationship between predatory capacity (no. prey items consumed in 7 days) and fecundity (no. eggs laid in 7 days) of *Amblyseius orientalis* on *Carpoglyphus lactis* **A** deutonymphs and **B** eggs

predator; however, the predation time was much longer than when preying on eggs. Besides body size, other factors might also affect predation, such as behavioral contact with the prey. Eggs obviously do not move, but predation of *A. orientalis* on mobile stages of *C. lactis* is relatively time-consuming, as the prey try to escape and avoid predation. Catching same size *C. lactis* adults was difficult for *A. orientalis*, as it was observed trying to grasp and prey on *C. lactis*. Apparently, the comparatively large body size, strong mobility and relatively thick tegument of *C. lactis* adults result in predation difficulty. Compared with *C. lactis* adults, the thin egg shell is easy to be pierced by the mouthparts of *A. orientalis*.

The development and reproduction of predators require crucial nutrients (Harvey et al. 2012; Williams and Roane 2007; Woods et al. 2020). Compared with high-protein prey, high-lipid prey had negative effects on the survival and reproduction of the web-building spider *Hylyphantes graminicola* (Wen et al. 2020). The predatory mite *Parasitus consanguineus* (Parasitidae), separately feeding on two prey species, the mushroom flies *Megaselia halterata* and *Lycoriella ingenua*, significantly differed in survival, female longevity and fecundity (Szlendak and Lewandowski 2009). When providing additive proteins or saccharides to prey mites in mass rearings, the phytoseiid predatory mite *Neoseiulus barkeri* presented higher fitness with shorter developmental durations and higher fecundity than those fed on basic diet (Huang et al. 2013). These results indicate that the food of the prey mites has a major impact on the nutritional value of the prey mites for predatory mites. In our study, *C. lactis* eggs could provide sufficient nutrition for *A. orientalis* reproduction, suggesting the developmental stage preference was not risky to predators in a long run. Our results are also consistent with a previous study that the eggs are sufficiently nutritious to support the predatory mite to complete reproduction and egg prey preference does not pose a survival risk to predators (Moghadasi et al. 2013). *Neoseiulus californicus* has been reported to prey eggs of five spider mite species which deposited on a variety of plants with similar performance (Gotoh et al. 2006). All this evidence indicates prey eggs are sufficient to support growth and development of the predatory mites.

In the current study, we found that fecundity of *A. orientalis* could be lower when providing higher density of *C. lactis* adults. It means that the ratio of prey and predator is critical during predatory mite rearing. As discussed above, we hypothesize that the energy supplied by the prey may be less than the energy consumed by *A. orientalis* during predation. Other explanations might be that the predators are deterred to prey *C. lactis* by an alarm pheromone or physical barrier. In mass-production, we should quantify prey density and strictly control the ratio of *C. lactis* to *A. orientalis* in inoculation to ensure the population growth of predatory mites. In short, our study elucidated the sensitive characteristics of *A. orientalis* to prey density, and provided a solution for stable population growth of predatory mites in mass rearing.

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Author contributions JW and YL: Investigation, formal analysis, Writing—original draft preparation, reviewing and editing. FS: Investigation and formal analysis. BZ: Conceptualization, Formal analysis, supervision, writing—original draft preparation, reviewing and editing. EW and XX: Validation, supervision and editing.

Declarations

Competing interests The authors declare no competing interests.

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