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# Development, survival, and reproduction of *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) feeding on fresh versus frozen eggs of *Tetranychus urticae* Koch (Acari: Tetranychidae)

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## Original research

### ABSTRACT

*Phytoseiulus persimilis* Athias-Henriot is an important biocontrol agent for controlling spider mites (*Tetranychus* spp). Rearing *P. persimilis* is relatively expensive due to the need to raise spider mites (e.g. *Tetranychus urticae* Koch), as prey, on live plants. Frozen eggs of *T. urticae* can be easily stored and used as a potential feed for laboratory rearing of *P. persimilis*. This study investigated the performance of frozen *T. urticae* eggs compared with fresh eggs as food for rearing *P. persimilis*. Under laboratory conditions, the survival, immature development, consumption, and oviposition of *P. persimilis* given frozen or fresh *T. urticae* eggs were observed. The influence of the dietary experience of *P. persimilis* females (i.e. fed on frozen or fresh spider mite eggs) on offspring's development and consumption was also examined. The survival and development of *P. persimilis* were not affected by the different diets. However, individuals fed on frozen *T. urticae* eggs had a reduced oviposition rate and an increased consumption compared with those given fresh eggs. The maternal dietary experience did not affect the development and consumption of *P. persimilis*. As the nutritional quality of *T. urticae* eggs is likely reduced during freezing, the frozen eggs of *T. urticae* may be inadequate compared with fresh eggs in rearing *P. persimilis*. Nevertheless, frozen eggs have the potential as supplementary food for *P. persimilis*. Future studies can investigate the performance of frozen *T. urticae* eggs on the lifespan and lifetime fecundity and in maintaining a population of *P. persimilis* in the field.

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**Keywords** *Phytoseiulus persimilis*; *Tetranychus urticae*; frozen egg; feed; development; oviposition

## Introduction

Many predatory mites from the family Phytoseiidae (Acari) are economically important and widely used biocontrol agents against phytophagous insect and mite pests (McMurtry *et al.* 2013; Liu and Zhang 2017; Bajda *et al.* 2022). Being one of the members, *Phytoseiulus persimilis* Athias-Henriot is a Type-I specialist predator that has been used mainly to control spider mites (*Tetranychus* spp.) for many decades (McMurtry *et al.* 2013; Bajda *et al.* 2022). Unlike generalist predatory mites, where non-prey resources such as pollen and artificial diets can be used as supplementary food to sustain their population, specialist predators are narrower in their food range (Pirayeshfar *et al.* 2020; Morris *et al.* 2021). The commercial and laboratory

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rearing of *P. persimilis* is more costly than the rearing of generalist predators since it often requires the maintenance of spider mites using various plants (Jedlickova 1992; Walzer and Schausberger 2015). Furthermore, collecting fresh spider mite eggs to feed *P. persimilis* for experimentation is sometimes difficult due to the presence of webbing, and short preservation time after collection since eggs will hatch within a few days (Razmjou *et al.* 2009).

Frozen prey has been used in the mass rearing of arthropod predators for biocontrol purposes and as supplementary food to aid their release into the field (Mohaghegh and Amir-Maafi 2007; Silva *et al.* 2013; Pirayeshfar *et al.* 2021). The use of frozen prey increased the food availability and preservation time for the predators, and also avoided the adverse impacts of using alive prey (Mohaghegh and Amir-Maafi 2007; Silva *et al.* 2013; Pirayeshfar *et al.* 2021). Previous studies have found that the frozen eggs and larvae of the storage mite *Tyrophagus putrescentiae* (Schrank) sustained the immature development and oviposition of generalist predatory mites *Amblyseius swirskii* Athias-Henriot (Pirayeshfar *et al.* 2020) and *Blattisocius mali* (Oudemans) (Pirayeshfar *et al.* 2021; Pirayeshfar *et al.* 2022). The bugs *Orius thripoborus* (Hesse) and *Orius naivashae* (Poppius) successfully developed and reproduced when given frozen eggs of the moth *Ephestia kuehniella* Zeller and fruit fly *Ceratitis capitata* (Wiedmann) (Bonte *et al.* 2017), so did predatory mites *Stratiolaelaps scimitus* (Womersley) (Xie *et al.* 2018). Thus, frozen prey can be a potential alternative food resource for rearing predators.

Eggs of the spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae), a ubiquitous polyphagous pest to greenhouse and field crops (Razmjou *et al.* 2009; Bajda *et al.* 2022), can sustain the development and oviposition of their primary predator *P. persimilis* (Blackwood *et al.* 2001). To simplify the laboratory mite rearing procedure, we examined whether the frozen eggs of *T. urticae* could support *P. persimilis*' development and reproduction. The performance of frozen eggs of *T. urticae* in rearing *P. persimilis* was evaluated and compared with mites reared on fresh eggs. We also examined the influence of the maternal dietary experience (fed on frozen or fresh eggs) on the offspring's development.

## Material and methods

### Mite rearing

The predatory mite *P. persimilis* was reared on its prey, spider mite *T. urticae*, and both were originally acquired from Bioforce Limited (Karaka, Auckland). The spider mites were cultured on bean plants (*Phaseolus vulgaris* L.) in a greenhouse (20 ± 2 °C) at Manaaki Whenua – Landcare Research, Auckland, and fed to *P. persimilis* at regular intervals. The cultures of *P. persimilis* consisted of detached bean leaves infested with spider mites on a black plastic sheet (approximately 15 cm × 10 cm). A water-saturated sponge elevated the black sheet in a plastic tray (22 × 22 × 3.5 cm) filled with water (Patel and Zhang 2017). *P. persimilis* colonies were kept in the laboratory at a temperature of 20 ± 1 °C, relative humidity of 65–75%, and 12h illumination.

### Experimental setups

Rearing cells consisted of three parts: a glass slide (20 × 15 mm and 1 mm thick) was placed on top; a plexiglass slide (25 × 20 mm and 1 mm thick) with a central hole 6 mm in diameter to host mites was placed in the middle; and a black cotton cloth (10 × 10 mm) was glued to the bottom of the plexiglass slide with non-toxic PVC glue (Think Creative) to allow air ventilation and increase contrast for observing the mites. The rearing cell was held together by two metal clips. Similarly aged eggs of spider mite *T. urticae* were collected from the culture and frozen at –18 °C for 48h before being used in the experiment. A restricted diet at the intermediate level (10 and 20 eggs daily) was given to *P. persimilis* mothers. A total of 20 spider mite eggs allowed over 90% survival of newly hatched *P. persimilis* to reach adulthood (Han *et al.* 2022), which was given to *P. persimilis* to sustain their immature development in this experiment.

## Experimental procedure

- 1) Dissimilarly aged gravid adult females (with large and inflated bodies) ( $n = 32$ ) were randomly selected from the culture as the testing subjects. The females were individually placed into the rearing cells and starved for 24 hours. Four treatments different in prey egg status and density were given to the females in the rearing cells at the end of their starvation period: 1) 10 fresh eggs, 2) 10 frozen eggs, 3) 20 fresh eggs, and 4) 20 frozen eggs. Spider mite eggs were replenished daily. Each treatment was replicated 8 times. Survival, oviposition, and daily egg consumption were recorded daily for five days. Oviposition rate was calculated as the number of eggs laid per female per day. Eggs laid by *P. persimilis* on the first day were removed and used in the second part of the experiment.
- 2) A two-factor analysis concerning maternal and own feed types was conducted. Eggs of *P. persimilis* collected from the rearing cells were assigned into two groups based on the maternal experience: mother fed on frozen or on fresh spider mite eggs. For each group, *P. persimilis* eggs were placed singly into new rearing cells. After hatching into larvae, two treatments were randomly given to the *P. persimilis* until the completion of their development: 20 fresh or 20 frozen spider mite eggs. Each treatment was replicated 10 times. The development, survival, and consumption of *P. persimilis* were observed twice a day at 8 am and 4 pm until the predator reached adulthood or died. After maturity, each adult predator was slide-mounted using Hoyer's medium (Walter and Krantz 2009). The sex of adult mites was distinguished under an interference-phase contrast microscope (Nikon Corporation, Japan) at 400 $\times$  magnification. All slides were deposited in New Zealand Arthropod Collection at Manaaki Whenua – Landcare Research, Auckland, New Zealand.

A climate incubator was used for the experiment (MIR-154, Sanyo, Japan). The temperature and photoperiod were controlled at  $25 \pm 1$  °C and 12L: 12D, respectively. The rearing cells were placed on an elevated plastic shelf in a plastic box ( $27 \times 27 \times 13$  mm) inside the incubator during the experiment. The plastic box contained saturated salt (NaCl) water beneath the shelf to maintain a relative humidity of approximately 80% (Lee *et al.* 2020).

## Statistical analysis

R version 4.0.5 (R Development Core Team 2021) with the package ARTool was used for statistical analysis (Elkin *et al.* 2021). Data were reported with means and standard error (SE). The aligned ranks transformation analysis of variance (ART ANOVA) was used to analyse oviposition, consumption, and immature development of *P. persimilis* due to the non-normal distribution of the data sets and multiple independent factors involved (i.e. a factorial design). Proportions were compared using the chi-square test. Statistically significant was considered when  $p < 0.05$ .

## Results

### Fecundity of mothers

The oviposition rate of *P. persimilis* females was significantly affected by the type (fresh or frozen) and density (10 or 20) of prey eggs (ART ANOVA:  $F = 34.06$ ,  $df = 1$ ,  $p < 0.001$  for type; and  $F = 87.28$ ,  $df = 1$ ,  $p < 0.001$  for density). A higher density of prey eggs allowed a higher reproduction of *P. persimilis* (Table 1). Furthermore, individuals of *P. persimilis* given fresh eggs had a significantly higher oviposition rate than those fed on frozen eggs at both egg densities (Table 1). Although limited individuals given frozen eggs died and all individuals from the fresh egg groups survived, the survival rate between individuals given the two feed types was not significantly different (Chi-square test:  $\chi^2 = 4.05$ ,  $df = 3$ ,  $p = 0.257$ ). The proportion of supplied eggs consumed did not differ between treatments, where *P. persimilis*

**Table 1** Mean ( $\pm$  SE) fecundity, consumption, and survival of *Phytoseiulus persimilis* females feeding on eggs of *Tetranychus urticae* at different densities. <sup>†</sup> Proportion of individual predators survived the five-day ovipositional period. <sup>‡</sup> Proportion of the given prey eggs consumed by the *P. persimilis*. <sup>§</sup> Individuals who died are not included in the analysis since they did not have the same ovipositional duration as the survived ones. Means followed by different letters indicate a significant difference (ART ANOVA post hoc comparison:  $p < 0.01$ ).

Egg type	Egg density	n	Survival <sup>†</sup>	Consumption (%) <sup>‡</sup>	Oviposition rate (egg/female/day) <sup>§</sup>
Fresh	20	8	100%	99.38 $\pm$ 0.63	3.33 $\pm$ 0.09 <sup>a</sup>
Fresh	10	8	100%	100.00 $\pm$ 0.00	1.58 $\pm$ 0.18 <sup>c</sup>
Frozen	20	8	87.50%	98.75 $\pm$ 1.25	2.54 $\pm$ 0.09 <sup>b</sup>
Frozen	10	8	75%	98.75 $\pm$ 1.25	1.07 $\pm$ 0.14 <sup>d</sup>

females consumed almost all eggs (ART ANOVA:  $F = 0.01$ ,  $df = 1$ ,  $p = 0.91$  for type; and  $F < 0.01$ ,  $df = 1$ ,  $p = 0.96$  for density).

### Consumption of offspring

The prey egg consumption of *P. persimilis* during immature development varied significantly between different prey egg types and predator sexes (ART ANOVA:  $F = 35.03$ ,  $df = 1$ ,  $p < 0.001$  for type; and  $F = 49.43$ ,  $df = 1$ ,  $p < 0.001$  for sex). In contrast, the maternal experience of *P. persimilis* did not affect individuals' consumption rate (ART ANOVA:  $F = 1.88$ ,  $df = 1$ ,  $p = 0.18$ ). Thus, data of different maternal experiences were pooled to examine the influence of egg type and sex-specific responses (Table 2). Females of *P. persimilis* consumed more prey than males for the same prey type. Individuals of *P. persimilis* of the same sex had a significantly higher prey consumption when given frozen eggs than those given fresh ones.

### Development of offspring

The maternal dietary experience and prey egg type did not influence the total developmental duration of *P. persimilis* (ART ANOVA:  $F = 1.17$ ,  $df = 1$ ,  $p = 0.29$  for maternal experience; and  $F = 1.62$ ,  $df = 1$ ,  $p = 0.21$  for type). Therefore, data were not subdivided based on the maternal dietary experience (Table 3). The developmental duration of *P. persimilis* was significantly affected by the predator's sex (ART ANOVA:  $F = 9.19$ ,  $df = 1$ ,  $p = 0.01$ ). When given fresh prey eggs, *P. persimilis* males had a significantly shorter duration (protonymph, deutonymph, and the total duration) than females (Table 3). Nevertheless, the developmental duration was similar between *P. persimilis* females and males fed on frozen prey eggs.

**Table 2** Mean ( $\pm$  SE) prey egg consumption of *Phytoseiulus persimilis* during immature development feeding on fresh or frozen spider mite (*Tetranychus urticae*) eggs. Means followed by different letters indicate a significant difference (ART ANOVA post hoc comparison:  $p < 0.01$ ).

Prey egg type	Sex	n	Total consumption
Fresh	Female	10	18.40 $\pm$ 0.56 <sup>b</sup>
Fresh	Male	10	11.00 $\pm$ 0.89 <sup>c</sup>
Frozen	Female	12	20.00 $\pm$ 0.00 <sup>a</sup>
Frozen	Male	8	17.63 $\pm$ 1.08 <sup>b</sup>

Discussion

The frozen eggs of *T. urticae* allowed the newly hatched *P. persimilis* to survive, reach adulthood, and sustain their oviposition for a set duration; however, as a feed for *P. persimilis*, the performance of frozen *T. urticae* eggs was not as adequate as that of fresh eggs. *P. persimilis* feeding on frozen spider mite eggs had a reduced oviposition rate and an increased consumption rate. Previous studies also found that *P. persimilis* reduced the number of eggs laid under food stress (Walzer and Schausberger 2015). Moreover, individuals would increase their consumption rate when given low-quality feed to sustain normal development (Kause *et al.* 1999). When reared in a greenhouse, the frozen larvae of *T. putrescentiae* were less competitive than fresh larvae or pollen in increasing the population size of the generalist phytoseiid *A. swirskii* (Pirayeshfar *et al.* 2020). Therefore, the nutritional value of eggs could be reduced by freezing. Future studies can investigate if the frozen *T. urticae* eggs alone can adequately sustain a population of *P. persimilis* outside a laboratory (e.g. greenhouse).

In contrast, frozen diets (eggs and larvae) of *T. putrescentiae* sustained a similar oviposition rate of *A. swirskii* compared with those fed with fresh diets or cattail pollen (*Typha angustifolia* L.) (Pirayeshfar *et al.* 2020). The lady beetles *Eriopis connexa* (Gemar) reared on frozen or fresh eggs of the moth *Spodoptera frugiperda* (Smith) had a similar weight at maturity and survival rate (Silva *et al.* 2013). Thus, the response of predators to fresh and frozen prey may be species specific or prey specific. For example, the predatory stinkbug *Andrallus spinidens* (F.) given fresh or frozen larvae of the moths *Galleria mellonella* (L.) and *E. kuehniella* differed in life history responses (Mohaghegh and Amir-Maafi 2007). When comparing fresh or frozen larvae of *G. mellonella* as diets, *A. spinidens* had similar developmental time and size at maturity. Conversely, the frozen larvae of *E. kuehniella* compared with fresh ones resulted in a shorter developmental duration and a smaller size of *A. spinidens*.

The developmental duration observed between *P. persimilis* feeding on fresh or frozen spider mite eggs was similar. Conversely, *A. swirskii* fed on frozen diets (eggs and larvae) of *T. putrescentiae* had a prolonged development compared with those fed with fresh diets or cattail pollen (*Typha angustifolia*) (Pirayeshfar *et al.* 2020). However, this is likely due to a difference in their developmental plasticity. Generalist phytoseiids were found to delay their development during food stress, while specialists accelerated their growth (Walzer and Schausberger 2011). Meanwhile, the nutritional difference between fresh and frozen eggs of *T. urticae* would be less likely to affect the development duration of the specialist phytoseiid *P. persimilis*.

In this study, *P. persimilis* males had a shorter development duration than females when fed on fresh *T. urticae* eggs. The size of *P. persimilis* is female-biased, but the time to reach adulthood was reported to be similar between females and males when reared under laboratory conditions (Laing 1968; Walzer and Schausberger 2011). In our previous study, 71 females and 53 males of *P. persimilis* that were given 20 fresh *T. urticae* eggs after hatching did not differ in their developmental time (Han *et al.* 2022). Therefore, the reason causing such a difference in this study is likely due to a randomly occurring individual variation.

Transgenerational effects can occur in mites and other animals (Li and Zhang 2019; Schausberger and Rendon 2022). For instance, the environmental influences, including food

**Table 3** Mean (± SE) immature developmental duration (in days) of *Phytoseiulus persimilis* feeding on fresh or frozen spider mite (*Tetranychus urticae*) eggs. † The survival rate (%) of *P. persimilis* to reach adulthood. The sample size is given in parentheses. ‡ The life stages of *Phytoseiulus persimilis* included are egg (E), larva (L), protonymph (P), deutonymph (D), and the total duration from egg to adult (E–A). Means followed by different letters indicate a significant difference within the same life stage (ART ANOVA post hoc comparison:  $p < 0.01$ ).

Egg type	Sex	Survival <sup>†</sup>	E <sup>‡</sup>	L <sup>‡</sup>	P <sup>‡</sup>	D <sup>‡</sup>	E–A <sup>‡</sup>
Fresh	Female	100(10)	1.60±0.13 <sup>a</sup>	0.65±0.12 <sup>a</sup>	1.13±0.11 <sup>a</sup>	1.16±0.11 <sup>a</sup>	4.52±0.14 <sup>a</sup>
Fresh	Male	100(10)	1.79±0.01 <sup>a</sup>	0.65±0.12 <sup>a</sup>	0.75±0.10 <sup>b</sup>	0.75±0.09 <sup>b</sup>	3.92±0.10 <sup>b</sup>
Frozen	Female	100(12)	1.55±0.13 <sup>a</sup>	0.83±0.09 <sup>a</sup>	1.14±0.10 <sup>a</sup>	1.08±0.07 <sup>a</sup>	4.58±0.22 <sup>a</sup>
Frozen	Male	100(8)	1.68±0.13 <sup>a</sup>	0.65±0.13 <sup>a</sup>	0.94±0.03 <sup>a,b</sup>	0.95±0.03 <sup>a,b</sup>	4.20±1.18 <sup>a,b</sup>

stress, social experience, and predation pressure, individuals face are passed on to provoke phenotypic variation in their offspring (Li and Zhang 2019; Schausberger and Rendon 2022). Nevertheless, the different diets (fresh or frozen eggs) given to *P. persimilis* mothers did not affect the consumption and development of their offspring. In this study, the feeding duration of mothers and the nutritional differences between fresh and frozen eggs might be insignificant in causing phenotypic variation in their offspring. *P. persimilis* eggs laid on the fifth day of the mothers after the start of the experiment might acquire a stronger maternal diet influence than eggs laid on the first day as used in this experiment, which may be tested in a future study.

Adult females selected in this experiment to observe oviposition were not similarly aged. Some *P. persimilis* females may be at the beginning of their oviposition, while others might be at the later stages. Randomisation in assigning treatments and the selection of a brighter coloured *P. persimilis* with an inflated body were practised to minimise such an influence. *P. persimilis* at the end of their ovipositional phase are darker and smaller than those at the beginning and mid phases (pers. obs.).

Frozen *T. urticae* eggs have the potential to be used as a supplementary feed for *P. persimilis*. Although freezing prey is helpful for the long-term storage of food resources, the nutritional quality of prey can be reduced during freezing (Silva *et al.* 2009). The performance of a better freezing technique such as flash freezing may be used in future studies to reduce moisture loss of the prey. Further studies should observe the lifespan and lifetime fecundity of *P. persimilis* feeding on frozen *T. urticae*.

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