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Immature development and survival of *Neoseiulus cucumeris* (Oudemans) (Acari: Phytoseiidae) on eggs of *Tyrophagus curvipenis* (Fain & Fauvel) (Acari: Acaridae)

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Original research

ABSTRACT

Establishment and maintenance of the natural enemy are critical for successful biological control of pests on plants without alternative food for predators. Great efforts have been devoted to exploring suitable supplementary food for predators. In this study, we evaluated the feasibility of using *Tyrophagus curvipenis* (Fain & Fauvel) (Acari: Acaridae) as a supplementary food source for *Neoseiulus cucumeris* (Oudemans) (Acari: Phytoseiidae) by investigating the survival and development of *N. cucumeris* at different prey densities with the influence of conspecifics. Furthermore, we investigated predation rates and their body size at adult emergence. The results showed that *N. cucumeris* developed from egg to adult in approximately six days. Survival rates of immature predators increased significantly with the given prey density. No significant difference in body size was found between the survived adults fed at different prey density, but the females were always larger than males. To conclude, *T. curvipenis* can be an excellent alternative food source for the biological control agent *N. cucumeris*.

Keywords predatory mite; factitious food; biological control; development; predation rate; conspecific

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Introduction

Phytoseiid mites (Acari: Phytoseiidae) are among the most important predatory species employed as biocontrol agents against agricultural pests such as phytophagous mites, thrips, and other small arthropods (van Lenteren 2012; Knapp *et al.* 2018; Rao *et al.* 2018). Many species in this family can be mass-reared with astigmatid mites (Acari: Astigmata) as factitious prey and are now commercially available (Gerson *et al.* 2003; Barbosa and Moraes 2015). *Neoseiulus cucumeris* (Oudemans) (Acari: Phytoseiidae), as one of the generalist predatory mites, is widely used (Jacobson *et al.* 2001; Zhang 2003; van Houten *et al.* 2005; McMurtry *et al.* 2013) ever since it was first used for biocontrol of thrips in 1985 (Knapp *et al.* 2018). It is well known worldwide for its biocontrol potential against a wide variety of pests such as mites, whiteflies, thrips, and psyllids of great agricultural and horticultural importance (Li & Zhang 2016, 2020; Kakkar *et al.* 2016; Li *et al.* 2017; Patel & Zhang 2017; Reitz *et al.* 2020).

The efficacy of biological control depends on many factors, including the characteristics of host plants and the availability of supplementary food. Biocontrol of thrips with *N. cucumeris* proved to be successful in sweet peppers: high densities of predators can be reached even in the absence of thrips because of pollen provided by the host plants (Ramakers 1988; 1990). However, by releasing predatory mites, some biological control programs were not successful when the pest population was small, or the plant could not offer the predators pollen as alternative food (Messelink *et al.* 2006; Skirvin *et al.* 2006). Therefore, in prophylactic control programs the key to successful biological control for the plants mentioned above is to provide the predators with supplementary food to maintain and enhance their population.

Some researchers suggested that dietary supplementary such as pollen, fungi (Skirvin *et al.* 2007), and the eggs of the Mediterranean flour moth *Ephestia kuehniella* Zeller (Lepidoptera: Phycitidae) (Delisle *et al.* 2015) should be provided to enhance the establishment and survival of predatory mites and to avoid cannibalism during the period of food scarcity (van Rijn *et al.* 2002; Nichols and Altieri 2004; Hoogerbrugge *et al.* 2008). Pollen is extensively studied as a dietary supplement for the natural enemy (e.g. Delisle *et al.* 2014; Liu & Zhang 2017; Pascua *et al.* 2020). However, many kinds of pollen from plants such as birch, hazel, and common cattail have been reported to have positive effects on the development and fecundity of secondary pests such as thrips (Van Rijn & Sabelis 1993; Hulshof *et al.* 2003; Skirvin *et al.* 2006). Although pollen can potentially reduce the efficiency of biological control, some studies, for example, Van Rijn (2002), have reported that *Typha latifolia* L. (Poales: Typhaceae) pollen is of benefit to the control of thrips even though it is edible for this pest, which contrasts with other kinds of pollens, which benefit thrips more than predators (Messelink *et al.* 2014). Therefore, the ideal alternative food for predatory mite should not only enhance the performance and boost the population of the predator but also not benefit the pest population. Although pollen, artificial diet, and moth eggs were feasible as a supplementary food source, exploring cost-effective alternative foods that can be commercially applied on a large scale in the field and greenhouses is a great challenge for biological control.

In this study, we explored a potential new alternative food, *Tyrophagus curvipes* Fain & Fauvel (Sarcoptiformes: Acaridae), which is a common mite found on several plants in Australia, France, Portugal, and New Zealand (Fan and Zhang 2007). This species closely resembles *Tyrophagus putrescentiae* (Schrank) in morphology but differs in biology. It is more commonly found on living plants rather than in stored products and no obvious damage caused by this species to plants has been observed up to now (Ye and Zhang 2014). It was found by Zhang (2003) that the population of *N. cucumeris* increased with the presence of *T. curvipes*. To evaluate the potential of *T. curvipes* as a food source for *N. cucumeris*, an experiment was conducted to investigate whether the predatory mites can complete their development when fed on eggs of *T. curvipes* at four prey densities. The following parameters in the immature stage were checked: survival rates, developmental time, predation rate and size at maturity. As it is well known that cannibalism is prevalent in generalist predatory mites when the food level is low, we further compared these responses with those of mites reared with conspecifics to understand how food levels and the presence of conspecifics influence the biology of predatory mites.

Material and methods

Mite culture

Neoseiulus cucumeris was obtained from Bioforce Ltd, New Zealand. The mite population was established in a petri dish with bran and *T. putrescentiae*. Dry yeast *Saccharomyces cerevisiae* was regularly added to this petri dish to provide enough food for *T. putrescentiae*.

Tyrophagus curvipes was collected from pepper leaves in a greenhouse in St Johns, Auckland, New Zealand. A colony of *T. curvipes* was established on a black plastic sheet (about 12 cm in diameter) with dry yeast, *Saccharomyces cerevisiae* (a common product used

in the bakery, produced by Goodman Fielder Limited, New Zealand) sprinkled on it. This sheet was placed on water-saturated paper tissue in a Petri dish (15 cm in diameter) to avoid mites escaping.

Both these populations were put into boxes with a saturated NaCl solution (common cook salt) to prevent the mites from escaping and maintaining a relatively suitable humidity (70 ± 10 % RH) for them.

Rearing units

The modified Munger cell (Ye and Zhang 2014) consisted of three plexiglass slides ($3.8 \times 2 \times 0.3$ cm) and a piece of black cloth. The middle slide, which was cone shaped (top diameter 10 mm and bottom diameter 7 mm), served as the cell, and the bottom slide was covered with a black cloth (to facilitate observation) and another plexiglass cone shape (to ensure the cell was well ventilated). The top slide was covered with a transparent glass fixed with two metal clips (Ye and Zhang 2014).

Collection of predator and prey eggs

The eggs of *N. cucumeris* were collected from the stock colony by providing to mites clean black cotton strings (about 1.5 cm in length) to facilitate oviposition. Next day, the black strings were checked, and the eggs produced within 24 h were collected using a small, fine hairbrush (size 00) and placed in a cell, individually or by pair.

The eggs of *T. curvipes* were collected from the lab population regardless of their ages. These eggs were transferred to each cell with a series of densities of 20, 40, 60, 80 eggs per cell. Before being used to feed *N. cucumeris*, they were frozen at -18 °C for 24 hours to ensure they would not hatch during the experiments.

Effects of prey density and conspecific

To clarify the effects of food level (i.e. density of prey eggs) and the presence of conspecific (i.e. *N. cucumeris* of the same age) on the development and survival of *N. cucumeris*, a full factorial design was employed where we crossed prey density (20, 40, 60, 80 eggs per cell) with the presence of a conspecific (Yes/No), generating eight treatments. Fifteen rearing units (replicates) were set up for each treatment. The cells were checked at 24-hour intervals to monitor the survival and development of *N. cucumeris*. The exuvium was used as an indicator to confirm that *N. cucumeris* developed to the next stage. The number of eggs consumed was determined every day, with shrivelled and dried eggs recorded as already being consumed. During the experiment, the dead conspecific was not replaced. The experiment was terminated when the remaining predator became adult or eventually died.

Effect of prey density and conspecific on the body size

Mites that successfully developed into adults were mounted on microscope slides using Hoyer's medium (Krantz and Walter, 2009). After one week in the oven (50 °C), they were observed under a compound microscope at $1000\times$ magnification and adult sex was determined. The length of the dorsal shield of predator mites was also measured as an indicator of body size. Mite specimens were vouchered in NZAC (Zhang 2018). All the experiments were conducted at a temperature of 25 ± 1 °C, a relative humidity of 80 ± 5 % RH, and a day/night cycle of 12:12 hours.

Statistical analyses

For statistical analyses, the parameters including developmental period and body size, which did not meet the assumption of normality, were subjected to Scheirer–Ray–Hare (SRH) test

with prey density and the presence/absence of conspecific as factors. Non-parametric Mann-Whitney test was also conducted to explore the effects of sex on body size without considering prey density and the presence of conspecifics. The number of prey consumed per predator was analysed by a two-way ANOVA with prey density and the presence/absence of conspecific as factors. The percentage data, namely the hatching rate and the survival rate of the predator mite, were analysed with a Chi-square test. The correlation between prey density and the daily consumption of eggs was performed with the R package “psych” version 1.7.5 (Revelle 2017). These analyses were conducted at the significance level of 0.05. R 3.4.1 (R Core Team 2017) was employed to conduct the data analysis.

Results

Survival rates of the immature stages

The hatching rate of *N. cucumeris* was at least 93% (Table 1). Neither the prey density ($\chi^2=2.024$, $df=3$, $P=0.567$) nor the presence of conspecifics ($\chi^2=0.451$, $df=1$, $P=0.501$) significantly affected the hatching rates of *N. cucumeris*. While survivorship of protonymphs was not significantly affected by prey density ($\chi^2=1.703$, $df=3$, $P=0.636$) and the presence of conspecifics ($\chi^2=0.000$, $df=1$, $P=1$), a significant difference was shown in their interaction ($\chi^2=4.42$, $df=1$, $P=0.035$). The survival rates of deutonymphs and adults dropped sharply because of food scarcity at the prey density of 20 and 40 per cell when compared with that of 60 and 80 prey per cell ($\chi^2=27.81$, $df=1$, $P<0.001$ for deutonymphs; $\chi^2=36.67$, $df=1$, $P<0.001$ for adults). Moreover, significantly lower survival rates were observed in deutonymphs and adults when predators were reared with conspecifics whatever the prey density ($\chi^2=5.31$, $df=1$, $P=0.021$ for deutonymphs; $\chi^2=6.70$, $df=1$, $P=0.01$ for adults).

Table 1 Survival rates (Mean $\pm$ se) of immature *Neoseiulus cucumeris* fed on *Tyrophagus curvipennis* eggs under different prey densities and conspecific presence/absence conditions at 25 ± 1 °C, relative humidity of 80 ± 5 % and day/night cycle of 12:12 hours.

Prey densities	Predator densities	N	Larvae	Protonymphs	Deutonymphs	Adults
20	one only	15	0.93 $\pm$ 0.07	0.79 $\pm$ 0.12	0.27 $\pm$ 0.26	0.00 $\pm$ 0.00
20	with conspecifics	30	0.96 $\pm$ 0.04	0.93 $\pm$ 0.05	0.16 $\pm$ 0.18	0.25 $\pm$ 0.43
40	one only	15	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00	0.43 $\pm$ 0.20	0.33 $\pm$ 0.33
40	with conspecifics	30	0.97 $\pm$ 0.04	0.93 $\pm$ 0.05	0.26 $\pm$ 0.17	0.14 $\pm$ 0.35
60	one only	15	1.00 $\pm$ 0.00	0.93 $\pm$ 0.07	0.71 $\pm$ 0.14	0.60 $\pm$ 0.20
60	with conspecifics	30	0.96 $\pm$ 0.04	0.93 $\pm$ 0.05	0.56 $\pm$ 0.13	0.29 $\pm$ 0.23
80	one only	15	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00	0.86 $\pm$ 0.10	0.83 $\pm$ 0.12
80	with conspecifics	30	1.00 $\pm$ 0.00	0.89 $\pm$ 0.06	0.60 $\pm$ 0.13	0.67 $\pm$ 0.15

Developmental periods of the immature stages

Neoseiulus cucumeris was able to complete its life cycle when it was offered enough *T. curvipennis* eggs. Approximately 6 days were needed to develop from egg to adult for the densities 60 and 80, for which it was possible to calculate this data (Table 2). At lower density, a too lower of deutonymphs survived. Prey density and conspecific presence (both $P>0.05$) did not significantly affect the developmental duration of larvae and protonymphs (for all prey densities). The duration of *N. cucumeris* deutonymphal stage was only compared at prey densities 60 and 80. The prey density significantly affected the deutonymphal stage, with the predatory mites reared at low prey density showing longer deutonymphal duration, with higher effects in presence of conspecifics ($H=11.445$, $P=0.010$; Table 2).

Table 2 The developmental duration (Mean± se) in days of different immature stages of *Neoseiulus cucumeris* fed on *Tyrophagus curvipes* eggs at different prey densities and conspecific presence/absence conditions at 25 ± 1 °C, relative humidity of 80 ± 5 % and day/night cycle of 12:12 hours.

Prey density	Predator density	Developmental time (days)				
		Egg	Larvae	Protonymph	Deutonymph ²	Immature
20	one only	1.83±0.13a ¹	1.00±0.11a	2.33±0.33a	--	--
20	with conspecifics	1.78±0.09a	1.06±0.07a	2.00±0.00a	--	--
40	one only	1.79±0.15a	1.00±0.13a	1.67±0.21a	--	--
40	with conspecifics	1.57±0.47a	0.96±0.07a	2.00±0.19a	--	--
60	one only	1.64±0.57a	0.85±0.12a	2.00±0.15a	2.00±0.00ab	6.00±0.43a
60	with conspecifics	1.79±0.54a	0.98±0.05a	2.46±0.27a	2.13±0.13a	6.38±0.97a
80	one only	1.72±0.49a	0.93±0.10a	2.08±0.19a	1.60±0.22a	6.20±0.30a
80	with conspecifics	1.70±0.48a	1.04±0.06a	2.27±0.21a	1.50±0.67b	6.00±0.17a

¹Values followed by different letters indicating the significant difference within the same column by Wilcoxon Test.

²Duration of deutonymphal and immature stages at the density of 20 and 40 were not shown in this table because no more than three predators successfully developed.

Predation rates of the immature stages

The consumption of *N. cucumeris* varied significantly according to the different life stages (Table 3). No predation was observed at larval stage. The number of *T. curvipes* eggs consumed by predator during the protonymphal stage ranged from 12.81 at prey density of 20 with two predators to 42.25 at 80 with only one predator. At the protonymphal stage, both the prey density and the presence of conspecific affected the predation of *N. cucumeris*. At a same prey density, the predator with conspecifics ate significantly fewer eggs than the lone predator ($F_{1,68}=13.46$; $P<0.001$). Significantly more eggs were consumed by the predator with increasing prey initial density ($F_{3,68}=10.33$; $P<0.001$). Deutonymphal predation was significantly affected by the presence of conspecifics ($F_{1,28}=13.37$; $P=0.001$), whereas, no influence was found among different prey densities ($F_{1,28}=1.52$; $P=0.229$). Significantly fewer prey were consumed by deutonymphs reared with conspecifics ($F_{1,28}=25.02$; $P<0.001$).

Table 3 Number of *Tyrophagus curvipes* eggs (Mean ±se) consumed by *Neoseiulus cucumeris* at different life stages under different prey densities and conspecific presence/absence conditions at 25 ± 1 °C, relative humidity of 80 ± 5 % and day/night cycle of 12:12 hours.

Prey density	predator	Prey consumed in each stage per predator			
		Larva	Protonymph	Deutonymph	Total Predation
20	one only	0	19.33±0.44 cd	-	-
20	with conspecifics	0	12.81±1.27 d	-	-
40	one only	0	23.25±1.66 bcd	-	-
40	with conspecifics	0	20.38±3.54 cd	-	-
60	one only	0	34.55±3.51 ab	20.60±2.98 ab	53.50±3.67 ab
60	with conspecifics	0	28.25±3.53 bc	10.75±1.43 b	38.50±7.29 c
80	one only	0	42.25±2.99 a	27.40±3.13 a	66.30±3.50 a
80	with conspecifics	0	28.59±2.53 bc	14.56±2.65 b	42.03±3.55 bc

Means followed by different letters indicate significant differences between means in the same column by Tukey's HSD tests.

The total number of eggs consumed by predator generally increased with prey density, but showed an interaction effect with the presence of conspecific for males (Fig. 1). For both males and females, the predation rates increased significantly with prey density ($R^2 = 0.781$, $P = 0.02$ for male, $R^2 = 0.771$, $P = 0.01$ for female). In the presence of conspecifics, female predation

Figure 1 Correlations between the initial prey density and the number of prey consumed by *Neoseiulus cucumeris* with/without conspecific: (A) males, (B) female. The dashed line, the dotted line, and the solid line represent linear fit to the mites reared in the absence of conspecific, reared with conspecific, male/female pooled together regardless of the presence of conspecific, respectively.

rates were lower than without and no significant correlation between prey density and predation rates was observed ($R^2 = 0.40$, $P = 0.29$ for female). On the contrary, the male predation rates were positively correlated with prey density ($R^2 = 0.98$, $P < 0.001$). When the male and female were pooled together regardless of the presence of a conspecific, there is a marginal effect of prey density on the number of prey consumed by the male ($R^2 = 0.472$, $P = 0.06$ for male) but a significant effect on the number of prey consumed by the female ($R^2 = 0.484$, $P = 0.04$).

Body size of *N. cucumeris* at maturity

Sexual size dimorphism was demonstrated in *N. cucumeris*: the body lengths of females were bigger than that of males regardless of prey densities and presence of conspecific ($W = 221.701$, $P < 0.001$; Fig. 2). No significant influence of the prey density ($H = 1.131$, $P = 0.288$) and the presence of conspecific on dorsal shield length was observed ($H = 0.084$, $P = 0.771$) without considering sex.

Discussion

In mass production of natural enemies, selecting cost-effective food sources is of critical importance in reducing cost in production. More recently, it was also suggested that provisioning natural enemies with supplementary food improved the outcome of biocontrol through enhancing the survival and reproduction of predatory mites when prey is scarce or unavailable (Vacacela Ajila *et al.* 2019). In this study, we determined the immature biology of *N. cucumeris* when they were offered a potential food source, *T. curvipes*, a mite species from the same family of the factitious prey *T. putrescentiae*. The results confirmed the usefulness of *T. curvipes* as a food source for *N. cucumeris*.

Our results showed that *N. cucumeris* was able to complete its life cycle when fed on eggs of *T. curvipes*, which was consistent with the previous report by Zhang *et al.* (2000) who showed that *N. cucumeris* can be reared on *T. putrescentiae*. By comparison, the *N. cucumeris* in our experiment developed almost as fast as those reared on cattail (*Typha latifolia*) pollen

Figure 2 Dorsal shield length (Mean $\pm$ se) of male and female of *Neoseiulus cucumeris* at different prey densities and conspecific presence/absence conditions. Asterisks indicate that there is a significant difference between male and female at the same density ($P < 0.05$). Dorsal shield length of males at the density of 60 with conspecifics were not shown because no males emerged at this density. NA is short for not available.

(Delisle *et al.* 2015; Nguyen *et al.* 2015), apple pollen (Delisle *et al.* 2014), and eggs of *Tetranychus urticae* Koch (Acari: Tetranychidae) (Li and Zhang, 2016), and much faster than those fed on the eggs of *E. küehniella* (Delisle *et al.* 2015) and artificial diet (Nguyen *et al.* 2015), suggesting that *T. curvipes* as a suitable food source for *N. cucumeris*.

According to this study, higher mortality in *N. cucumeris* was observed when the predatory mites were reared together with their conspecifics. One reason is that when the predators encountered food scarcity, some of them died of starvation. Higher mortality can also be attributed to cannibalism, a common phenomenon in the animal kingdom where a species attacks, kills, and even eats its conspecifics. During this experiment, cannibalism occurred when deutonymph and protonymph attacked the protonymph and larvae, respectively. Cannibalism was reported in previous studies (Schausberger & Croft 2000; Schausberger 2003), that indicated that smaller *N. cucumeris* individuals such eggs, larvae, and protonymphs were often cannibalized by larger ones such as protonymphs, deutonymphs, and females. This only occurred when prey was scarce or absent, indicating that *N. cucumeris* could discriminate the eggs of *T. curvipes* and immature of *N. cucumeris*, preferring the former.

Neoseiulus cucumeris larvae, as already documented, developed and survived without feeding (Zhang and Croft 1994). Reduced predation rates of *N. cucumeris* was witnessed when they were reared with conspecifics. A plausible explanation is that the competition between them reduced their consumption rates. In our previous study, immature *N. cucumeris* consumed about 107 to 150 *T. urticae* eggs in more than six days to develop into adults (Li and Zhang, 2016). In comparison, *N. cucumeris* intook only 39 to 65 *T. curvipes* eggs per predator, about half of the spider mite eggs in the immature stage and developed a little bit faster. This indicates that the latter is a more optimal food source for this predatory mite.

Food availability is an important factor affecting both life-history characters (e.g. Mikolajewski *et al.* 2005; Carabio *et al.* 2017). Previous studies with other predators showed that food limitation affected body size at maturity and development of many species. Nevertheless,

the response of these life-history traits varied greatly with species. For example, it was reported that prey (*T. urticae* egg) limitation did not influence the development of *Amblyseius andersoni* Chant (Acarina: Phytoseiidae) but accelerated that of *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) and *Neoseiulus californicus* McGregor (Acari: Phytoseiidae) (Walzer and Schausberger 2011). For all these mite species, female body size at maturity increased significantly with the increasing density of prey (Walzer *et al.* 2004). However, in this study, we found that both development and body size were not significantly affected by prey density. This distinct response to food limitation may result from their difference in larval feeding plasticity. For *N. cucumeris*, no food is required for the development of larvae to protonymphs and the larvae do not feed even when food is provided (Zhang and Croft 1994; also in this study), the lower food requirement and high plasticity making this species more resilient to food stress than other species. Moreover, the predatory mites showed very short development time without any other cost such as body size, indicating the eggs of *T. curvipes* are highly nutritious for them. This might mask the adverse effects of food shortage. Some limitations of this study such as the limited range of prey egg density and the long interval of observation might also contribute to the non-significant difference in development duration and body size of *N. cucumeris*.

In conclusion, the predatory mite *N. cucumeris* performed very well with *T. curvipes* eggs as food, developing fast and resilient to food limitation. Compared with other suggested supplementary foods such as pollen, artificial diets, and Mediterranean flour moth eggs, *T. curvipes* has some advantages over other alternative diets for *N. cucumeris* and other predators. *N. cucumeris* population could possibly establish readily as long as it was provided with *T. curvipes* when the pest density was low at the very beginning of biological control or when pollen is rare on the host plants. Furthermore, *T. curvipes* has not been observed to cause any damage to the plants (Ye & Zhang 2014). Thus, releasing *T. curvipes* is cost-effective in terms of investment (Xie *et al.* 2018). However, this is the first study on this topic. Taking the complex factors in the field and greenhouses into account, further studies on other aspects of the interaction of this predator-prey interaction in field or semi-field experiments are needed to explore further whether *T. curvipes* can be used optimally as an alternative food for biological agent *N. cucumeris*.

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Declaration of interest statement

No potential conflict of interest was reported by the authors.

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