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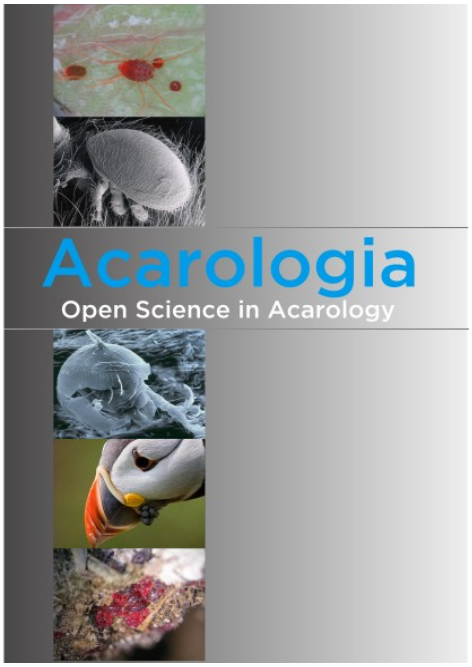

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Previous volumes (2010-2021): 250 € / year (4 issues)
Acarologia, CBGP, CS 30016, 34988 MONTFERRIER-sur-LEZ Cedex, France
ISSN 0044-586X (print), ISSN 2107-7207 (electronic)

The digitalization of Acarologia papers prior to 2000 was supported by Agropolis Fondation under the reference ID 1500-024 through the « Investissements d'avenir » programme (Labex Agro: ANR-10-LABX-0001-01)

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Effects of temperature on life table parameters of a newly described phytoseiid predator, *Neoseiulus neoagrestis* (Acari: Phytoseiidae) fed on *Tyrophagus putrescentiae* (Acari: Acaridae)

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Original research

ABSTRACT

The predatory mite, *Neoseiulus neoagrestis* Khaustov & Döker (Acari: Phytoseiidae), recently described as new species for science, but seems to have a great potential to be used in biological control. In this study, as a first step to determine the optimum temperature (between three temperatures tested) for its mass rearing, we studied its biological characteristics and life table parameters fed on *Tyrophagus putrescentiae* (Schrank) (Acari: Acaridae) at three different temperatures (20, 25, and 30 °C) under laboratory conditions. Results show that immature development, oviposition period as well as reproductive potential of this predator are significantly vary depending on the temperature. The immature developmental times and adult longevity for females, are ranged between 5.42 to 10.42 days and 39.88 to 74.12 days, based on the temperature, respectively. Average total number of eggs laid by per female at 25 °C (62.29) and 30 °C (58.65) are more or less similar but higher than that determined at 20 °C (41.46). The highest net reproductive rate (R_0) is 29.066 (offspring/individual) at 25 °C. In addition, the highest intrinsic rate of natural increase (r) is 0.241 day^{-1} at 30 °C. Furthermore, the highest finite rate of increase (λ = 1.272 day^{-1}), and the shortest mean generation time (T = 13.416 days) and doubling time (DT = 2.874 days) are also determined at 30 °C. Our results demonstrate that *N. neoagrestis* successfully completed immature development, and can survive and reproduce at all tested temperatures. Nevertheless, a series of biological characteristics of this predator are differentially affected by the temperature. Finally, 25 °C and 30 °C seems to be more suitable for mass rearing of this predator on *T . putrescentiae*. Further studies should be conducted to determine biological characteristics and life table parameters of *N. neoagrestis* on several important pests such as spider mites, whiteflies and thrips.

Keywords Phytoseiidae; Acaridae ; mass rearing; life table parameters; temperature

Received 05 October 2022
Accepted 22 December 2022
Published 09 January 2023

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Academic editor
Marčić, Dejan

https://doi.org/10.24349/n3ej-nn6s

ISSN 0044-586X (print)
ISSN 2107-7207 (electronic)

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How to cite this article Moradi M. *et al.* (2023), Effects of temperature on life table parameters of a newly described phytoseiid predator, *Neoseiulus neoagrestis* (Acari: Phytoseiidae) fed on *Tyrophagus putrescentiae* (Acari: Acaridae). **Acarologia 63(1): 31-40.** https://doi.org/10.24349/n3ej-nn6s

INTRODUCTION

Predatory mites belonging to the family Phytoseiidae (Acari: Mesostigmata) are important biological control agents of some phytophagous mites and insects such as tetranychoid and eriophyoid mites, whiteflies, thrips and free-living nematodes (McMurtry *et al.* 2013). Although about 2800 species of phytoseiid mites are currently known, only a few have been studied with regard to their potential as biological control agents (McMurtry *et al.* 2013; Tsolakis *et al.* 2013; Knapp *et al.* 2018). Among them *A. swirskii* Athias-Henriot, *Neoseiulus californicus* (McGregor), *N. cucumeris* (Oudemans) and *Phytoseiulus persimilis* Athias-Henriot are mass produced predators and commercially available for the biological control of spider mites, thrips, and whiteflies (Knapp *et al.* 2018). Despite many successful cases obtained by using the aforementioned predators in the biological control, the determination of natural or naturalized populations of phytoseiid mites are of considerable importance due to their adaptations to local environmental conditions (Gerson 2014). The cosmopolitan mould mite, also known as copra or cheese mite, *Tyrophagus putrescentiae* (Schrank) (Acari: Acaridae) is found in a variety of habitats including stored products, decaying organic matter, plant seeds, medicinal plants and mushroom beds (Eraky 1995; Chmielewski 1999). In addition, *T. putrescentiae* is a common pest of stored foods with relatively high fat and protein content, such as cheese, copra, dried egg, groundnuts, ham, herring meal and linseed as well as dried bananas, wheat spillage, oats, barley and flour (Hughes 1976; Mullen and O'Connor 2009). Besides, it can also colonize in human dwellings especially in areas with relatively high humidity and temperature such as in carpets, sofas, and blankets, and considered as one of the main sources of allergens in house dust worldwide (Fernandez-Caldas *et al.* 1990). Furthermore, *T. putrescentiae* is one of the most important food sources which is commonly used for mass rearing of generalist phytoseiid mites, due to its low cost of production on inexpensive food sources such as bran, yeast, and flour (Vangansbeke *et al.* 2014; Barbosa and de Moraes 2015). Knowledge about the biological characteristics and life table parameters of phytoseiid predators, on the other hand, is considered being one of the most important steps for evaluation of their potential on preys (Tsolakis *et al.* 2016; Ben Chaaban *et al.* 2018; Tsolakis *et al.* 2019; Mortazavi *et al.* 2022). Previous studies revealed that the life table parameters of phytoseiid mites are differentially affected by several important factors such as temperature, relative humidity, host plant, prey species, age and pesticide application (Sugawara *et al.* 2017; Tsolakis *et al.* 2019; Ersin *et al.* 2021).

In this study, as a first step to determine the optimum temperature among the three temperatures tested (20, 25, and 30 °C) under laboratory conditions, we studied biological characteristics and life table parameters of *Neoseiulus neoagrestis* (as a recently described phytoseiid species), fed on *T. putrescentiae* with the aim of mass rearing.

Material and methods

Mite colonies

Initial colony of *T. putrescentiae* were obtained from soil samples collected from Tyumen, Russia. The mites were extracted by using Berlese-Tullgren funnels into tap water and transferred into the plastic containers (12 X 8 X 4 cm in size) sealed with a mesh (< 100 micrometers) on the lid for ventilation. Sufficient amount of wheat bran was provided as a food source for the mites. The predatory mite, *Neoseiulus neoagrestis* was collected from moss in Sochi, Russia (Khaustov *et al.* 2022). The stock culture of the predator was maintained in the plastic containers described above using *T. putrescentiae* as a food source. The prey and the predator rearing units were kept separately in incubators (SANYO Electric Biomedical co., Japan) at 25 °C and 90 ± 5% RH and 12: 12 L: D. The predatory mites were reared for several generations, before being used in experiments.

Immature development and survival

The immature development and survival of the predators were individually observed by using transparent plastic containers (3 cm in diameter and 1.8 cm deep). The lid of the experimental test units was drilled (2 cm in diameter) and covered with a fine mesh (<100 micrometers in size) to allow for ventilation. Two to three gravid females of the predator obtained from the stock culture were transferred to experimental arenas using a brush (number 000) under a stereo microscope (Zeiss, Stemi-305, Germany). Subsequently, egg laying was observed in the arenas at two-hour intervals. The eggs (when there was more than one egg) and adult females of *N. neoagrestis* were removed from the experimental arenas using a brush, and only one egg was kept in each arena. Sufficient amounts of *T. putrescentiae* mixed with bran was provided as a food source for the predators at daily intervals. The duration of the immature stages including egg, larva, protonymph, and deutonymph were separately determined at three temperatures (20, 25 and 30 °C, and a constant humidity 90 ± 5% RH and photoperiod 12: 12 L: D), based on the observations conducted at 12-hour intervals.

Adult survival and fecundity

The female and male individuals successfully reached the adult stages were individually monitored at daily intervals to obtain the preoviposition, oviposition, and postoviposition periods as well as the number of eggs produced, in the same experimental units and conditions described earlier. In order to increase the mating likelihood, each female was paired with two males that were obtained from the stock culture. The observations were finalized when the last individual of the original cohort had died. The sex ratio of the offspring was determined by rearing all eggs deposited by the predator‘s females to adulthood.

Life table parameters

The life table parameters of *N. neoagrestis* were estimated based on age-stage, two-sex life table analysis (Chi and Liu 1985). The age-stage-specific survival rate (s_{xj}) (where x and j are age and stage, respectively); the age-stage specific fecundity (f_{xj}) of adult females; the age-specific survival rate (l_x); the age-specific fecundity (m_x), adult preoviposition period (APOP), total preoviposition period (TPOP), and the life table parameters including the net reproductive rate (R_0), intrinsic rate of increase (r), finite rate of increase (λ), gross reproductive rate (GRR), DT doubling time and mean generation time (T) were calculated in TWOSEX-MSChart computer program (Chi, 2020). The standard errors were estimated by using bootstrap method, and the differences were determined by using paired bootstrap test (Efron and Tibshirani 1993).

Results

Immature development and survival

Although immature stages of *N. neoagrestis* successfully reach adulthood at all temperatures tested, the temperature had significant effect on immature development and survival of this predator (Table 1). In general, the duration of immature stages obtained at each temperature was significantly different from each other for both sexes except for larva duration for females. The larva duration of females obtained at 25 °C and 30 °C were similar but significantly shorter than those obtained at 20 °C. In addition, no significant difference was detected between immature survival rates among the temperatures tested.

Adult survival and fecundity

Similar to the immature development, total pre-oviposition, adult pre-oviposition, and oviposition periods (TPOP, APOP, OP), and longevity of *N. neoagrestis* fed on *T. putrescentiae*

Table 1 Immature development (mean±SE in days) and survival rate (mean±SE) of *Neoseiulus neoagrestis* fed on *Tyrophagus putrescentiae* at three different temperatures under laboratory conditions.

		20ºC	25ºC	30ºC
Egg	♀	2.92±0.18a*	1.86±0.06b	1.50±0.11c
	♂	2.67±0.22a	1.81± 0.09b	1.55±0.16c
Larva	♀	1.69±0.11a	0.96±0.04b	0.81±0.07b
	♂	1.56±0.15a	0.69±0.09c	0.95±0.14b
Protonymph	♀	2.38±0.13a	1.89±0.06b	1.58±0.12c
	♂	2.11±0.07a	1.81±0.09b	1.60±0.30c
Deutonymph	♀	3.42±0.12a	2.36±0.11b	1.54±0.12c
	♂	3.28±0.12a	2.25±0.13b	1.60±0.21c
Egg to adult	♀	10.42±0.17a	7.07±0.12b	5.42±0.19c
	♂	9.61±0.33a	6.56±0.15b	5.70±0.62c
Survival rate		0.73±0.08	0.73±0.08	0.77±0.07

*Means within a row followed by different letters are significantly different according to the paired bootstrap test with 100,000 replicates (P<0.05).

were significantly affected by the temperatures tested (Table 2). The shortest TPOP (6.81 days), APOP (1.38 days) and OP (20.27 days), and female (39.88 days) and male (47.20 days) longevities were detected at 30 °C. In addition, these values were significantly different than those obtained at 20 °C and 25 °C (except APOP, and OP). Furthermore, the longevity of females was shorter than males in all temperatures tested. The highest total number of eggs (62.29) laid by per female was determined at 25 °C and this value is significantly different from that obtained at 20 °C (41.46), but similar to that obtained at 30 °C (58.65).

Table 2 Total pre-oviposition, adult pre-oviposition and oviposition periods (TPOP, APOP, OP), longevity, (mean±SE in days) and fecundity (mean±SE) of *Neoseiulus neoagrestis* fed on *Tyrophagus putrescentiae* at three different temperatures under laboratory conditions.

	20ºC	25ºC	30ºC
TPOP	14.35±0.16a	9.43±0.25b	6.81±0.24c
APOP	3.92±0.29a	2.36±0.20ab	1.38±0.14b
OP	37.69±3.53	31.75±2.41	20.27±1.80
Female total longevity	74.12±5.29a	62.21±4.74a	39.88±2.77b
Male total longevity	90.50±3.68a	73.56±3.00b	47.20±3.17c
Total fecundity	41.46±3.61b	62.29±5.26a	58.65±5.03a

*Means within a row followed by different letters are significantly different according to paired bootstrap test with 100,000 replicates (P<0.05).

Life table parameters

The highest intrinsic rate of increase (r= 0.241 day^{-1}), finite rate of increase (λ= 1.272 day^{-1}), and the shortest mean generation time (T= 13.416 days), and doubling time (DT= 2.874 days) obtained at 30 °C, and these values are significantly different from those obtained at two other temperatures (Table 3). In contrast, no significant difference was detected between the net reproductive rates (R_0), and gross reproductive rates (GRR) among the temperatures. However, numerically higher values were determined at 25 °C (R_0= 29.066 offspring/individual, GRR= 41.543 offspring/individual).

Table 3 Population parameters (r, intrinsic rate of increase; λ, finite rate of increase; R_0, net reproductive rate; T, mean generation time; GRR, gross reproductive rate; and DT doubling time of *Neoseiulus neoagrestis* fed on *Tyrophagus putrescentiae* at three different temperatures under laboratory conditions (mean±SE in days).

	20ºC	25ºC	30ºC
r (day^{-1})	0.099±0.009c*	0.172±0.015b	0.241±0.020a
λ (day^{-1})	1.104±0.010c	1.187±0.017b	1.272±0.026a
R_0 (offspring/individual)	17.966±4.043	29.066±6.143	25.416±5.719
T (day)	29.094±1.154a	19.577±0.824b	13.416±0.487c
GRR (offspring/individual)	25.873±5.168	41.543±7.785	33.943±6.930
DT (day)	6.981±0.750a	4.027±0.380b	2.874±0.270c

*Means within a row followed by different letters are significantly different according to paired bootstrap test with 100,000 replicates (P<0.05).

The age-stage survival rate curves (s_{xj}) show the probabilities of survival rate of newly emerged *N. neoagrestis* individuals to age x and stage j (Figure 1A). The variations in development rates resulted in overlaps between different developmental stages at three different temperatures. The highest survival rates of females and males are 43.33 and 0.30 at 20 °C, 0.46 and 0.26 at 25 °C, 0.43 and 0.33 at 30 °C, respectively.

The age-specific survival rate (l_x) showed more or less a similar pattern of gradual decline during the development at three different temperatures tested, but (l_x) declined faster at 30 °C compared to the other two temperatures (Figure 1B). The first oviposition occurred on the days 13th, 7th, and 6th at 20, 25 and 30 °C, respectively. The highest values for m_x and $l_x m_x$ were observed at 30 °C.

The age-stage-specific life expectancy (e_{xj}) shows the future expected life span of an individual of age x and stage j (Figure 2A). The life expectancies of a newly emerged individual were 60.59, 49.49 and 33.59 days at 20, 25 and 30 °C, respectively. In general, the age-stage-specific life expectancy (e_{xj}) values of adult females of *N. neoagrestis* are lower than those of males at all temperatures tested. In addition, the (e_{xj}) values obtained at 30 °C was lower compared to the other two temperatures, which demonstrated that life expectancies might be shorter at high temperatures. The highest age-stage-specific reproductive values (v_{xj}) of *N. neoagrestis* fed on *T. putrescentiae* were 9.75 on 20th day, 11.50 on 11th day, and 13.96 on 8th day, at 20 °C, 25 °C, and 30 °C, respectively (Figure 2B).

Discussion

Temperature is known to be one of the most important stressors for all organisms including insect and mite species (Wojda 2017). Many previous studies reported that the biological characteristics and life table parameters of phytoseiid mites, one of the most important groups of predators for their potential to be used in biological control, are also influenced by the temperature (Tsoukanas *et al.* 2006; Vangansbeke *et al.* 2013; Yazdanpanah *et al.* 2022). Therefore, the determination of a suitable temperature for the development and reproduction of phytoseiid mites is the first step for their mass rearing and usage in biological control.

Neoseiulus neoagrestis is a recently described phytoseiid species and nothing is known concerning its biological parameters on its prey (Khaustov *et al.* 2022). However, our preliminary observations showed that it might be a promising candidate for the biological control of spider mites. Therefore, this study includes the first and preliminarily data regarding biological characteristics and life table parameters of *N. neoagrestis* on *T. putrescentiae* at different temperatures. According to results, both males and females of the predator could develop to adults on the prey *T. putrescentiae* at all temperatures tested. Nevertheless, several biological characteristics, especially adult longevity and fecundity as well as life table parameters were differentially affected by the temperature.

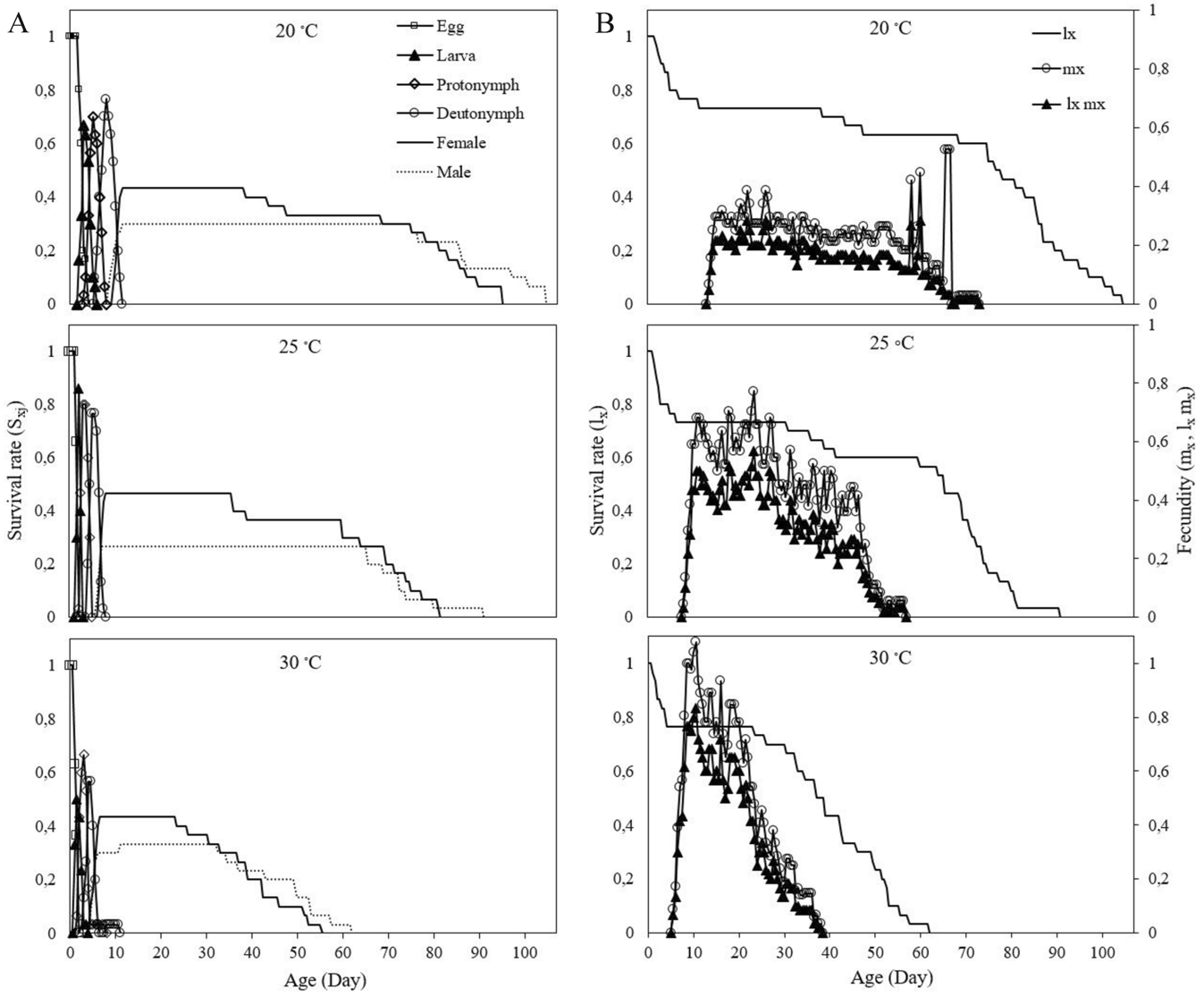

Figure 1 A – Age-stage specific survival rate (*Sxj*) of *Neoseiulus neoagrestis* fed on *Tyrophagus putrescentiae* at three different temperatures under laboratory conditions; B – Age-specific survival rate (l_x), age-specific fecundity (m_x), and age-specific maternity ($l_x m_x$) of *Neoseiulus neoagrestis* fed on *Tyrophagus putrescentiae* at three different temperatures under laboratory conditions.

The total developmental periods of females and males of the predator obtained at 25 °C were 7.07 days for females and 6.56 days for males. The total developmental period of *N. neoagrestis* females was higher than those obtained for *A. eharai* Amitai & Swirski (4.9 days), *A. swirskii* (5.1 days) and *N. cucumeris* (5.9 days) fed on *Carpoglyphus lactis* (L.) (Acari: Carpoglyphidae) at the same temperature (Ji *et al.* 2015). According to Ibrahim and Palacio (1994) the total immature developmental period for both sexes of *N. longispinosus* fed on *Tetranychus urticae* Koch (Acari: Tetranychidae) was 4.3 days at 25 - 28 °C. It was 9.2 days for males and 9.6 days for females of *N. barkeri* fed on *Oligonychus afrasiaticus* (McGregor) (Acari: Tetranychidae) at 25 °C (Negm *et al.* 2014). However, these values were decreased to 8.5 and 9 days for males and females, respectively, when the temperature increased to 35 °C. In addition, Gotoh *et al.* (2004) reported the total immature developmental period for both sexes of *Neoseiulus californicus* (McGregor) fed on *T. urticae* as 7.2 and 4.3 days at 20 °C and 25 °C, respectively and 3.3 days for males and 3 days for females at 30 °C. In addition, the immature

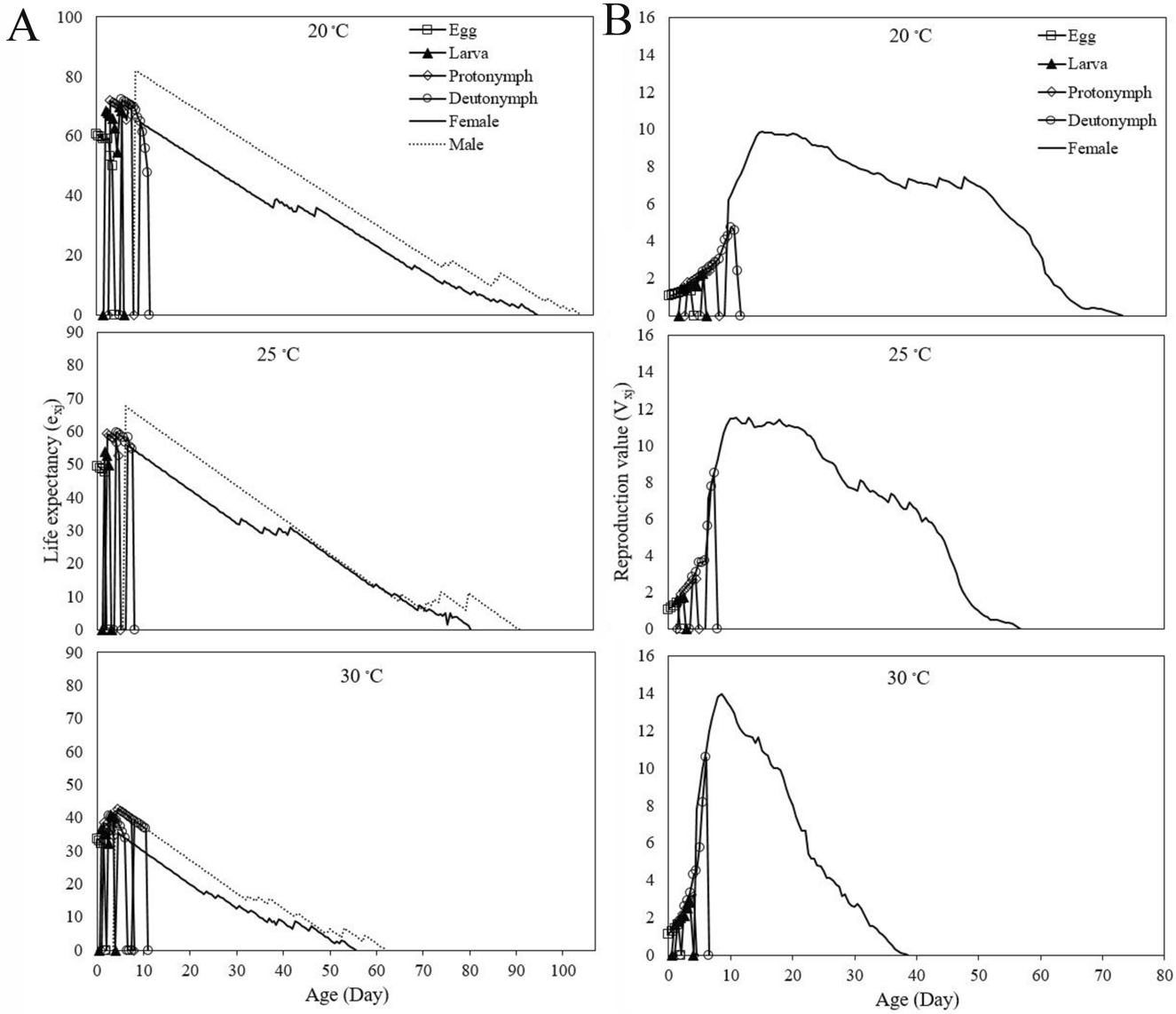

Figure 2 A – Age-stage-specific life expectancy (e_{xj}) of *Neoseiulus neoagrestis* fed on *Tyrophagus putrescentiae* at three different temperatures under laboratory conditions; B – Age-stage-specific reproductive value (v_{xj}) of *Neoseiulus neoagrestis* fed on *Tyrophagus putrescentiae* at three different temperatures under laboratory conditions.

development of *Neoseiulus neoagrestis* males were shorter than those of the females. Similar results were reported in a number of phytoseiid species such as *N. barkeri* Hughes (Jafari *et al.* 2011), *Euseius finlandicus* (Oudemans) (Broufas and Koveos 2001), *Phytoseius plumifer* (Canestrini & Fanzago) (Louni *et al.* 2014) and *N. longispinosus* (Evans) (Rahman *et al.* 2013).

Percent survival rate from egg to adult at all temperatures tested, was lower in present study than those reported for *N. longispinosus* by Rahman *et al.* (2013) and for *N. californicus* by Gotoh *et al.* (2004). Ji *et al.* (2015) reported that the percent immature survival for *N. cucumeris* at 25 °C was 58.54, which is also lower when compared to the results of the present study.

Similar to immature development, adult longevity was also found to be significantly different between constant temperatures for both males and females. A similar trend has been reported for two other *Neoseiulus* species, *N. longispinosus* (Rahman *et al.* 2013) and *N.*

barkeri (Xia *et al.* 2012). In another study, Negm *et al.* (2014) reported that the longevity of *N. barkeri* fed on *O. afrasiaticus* at 25 °C was 35.6 and 30.3 days for females and males, respectively. In addition, average female longevity for *N. cucumeris* and *Amblyseius swirskii* fedding on *C. lactis* were 67.3 and 45.7 days, respectively (Ji *et al.* 2015).

The preoviposition, the oviposition and adult longevity determined in this study are generally higher than those reported for *N. longispinosus* (Rahman *et al.* 2013) and *N. californicus* (Gotoh *et al.* 2004).

The highest total number of the eggs laid by the female *N. neoagrestis* was 62.29 at 25 °C followed by 58.65 at 30 °C and 41.46 at 20 °C. *Neoseiulus californicus* fed on *Panonychus ulmi* (Koch) (Acari: Tetranychidae) produced 51.88, 63.94 and 48.74 at 20, 25 and 30 °C, respectively (El Taj and Jung 2012). However, much lower values, 41.6, 38.4 and 28.4 eggs at 20, 25 and 30 °C, were reported for the same species fed on *Tetranychus urticae* (red form), respectively (Gotoh *et al.* 2004). Bond (1989) reported that the total number of the eggs laid by *N. barkeri* females fed on *Thrips tabaci* Lind. (Thysanoptera: Thripidae) was 47.1 at 25 °C. Jafari *et al.* (2011) found that *N. barkeri* fed on *T. urticae* produced 38.62 eggs during its lifespan. In addition, Ibrahim and Palacio (1994) determined the total number of eggs laid by *N. longispinosus* 50.7 at 25-28 °C. These results clearly showed that the effects of temperature on egg production of phytoseiid mites are variable depending on species.

In addition to other biological characteristics, the life table parameters are also variable and influenced by the temperature. The intrinsic rate of increase ($r = 0.241$ day^{-1}) and finite rate of increase ($\lambda = 1.272$ day^{-1}) determined at 30 °C in our study were more or less similar to those reported for *N. longispinosus* ($r = 0.268$ day^{-1}, $\lambda = 1.3$ day^{-1}) at the same temperature (Rahman *et al.* 2013). In contrast, the r value (0.172 day^{-1}) determined at 25 °C in the current study, was lower than those determined for *A. eharai* (0.253 day^{-1}), *A. swirskii* (0.232 day^{-1}) and *N. cucumeris* (0.212 day^{-1}) fed on *C. lactis* (Ji *et al.* (2015). The net reproductive rate ($R_0 = 29.06$ offspring/individual) determined at 25 °C in the current study, was more or less similar to that reported for *N. longispinosus* preying on *T. urticae* (R_0 29.4 offspring/individual) (Kolodochka 1983). However, the mean generation times (T=13.41 - 29.09 days) of *N. neoagrestis* were longer than those determined for *N. longispinosus* (9.0 - 9.8 days) (Ibrahim and Palacio 1994; Kolodochka 1983). The doubling time determined (DT= 2.87 days at 30 °C) in the present study, is also shorter than that reported for *N. longispinosus* (3.2 days) (Rahman *et al.* (2013), but lower than that found for *N. californicus* (2.37 days) (El Taj and Jung 2012).

In conclusion, this study reports, the biological characteristics and life table parameters of a newly described and a potential predatory mite to be used in biological control, for the first time. The results showed that the performance of *N. neoagrestis* seems to be better at 25 °C and 30 °C compared to 20 °C. By its fecundity, *N. neoagrestis* is close to such successful biocontrol agents as *N. californicus* and *N. cucumeris*. In addition, a prolonged lifespan potentially lets the predator persist in greenhouses for two months. Further studies should be conducted to determine biology of this predator on agriculturally important pests including spider mites, thrips and whiteflies. In addition, the efficacy of the predator against aforementioned pests should also be determined in field and greenhouse conditions.

Acknowledgements

The present research was supported by the grant from the Russian Science Foundation, project number 20–64–47015.

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