

Effect of cold storage on development and demographic parameters of *Scolothrips longicornis* fed on two-spotted spider mite

Hajar Pakyari 

Department of Entomology, Takestan Branch, Islamic Azad University, Takestan, Iran

Research Paper

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Keywords:Biocontrol; life table; low-temperature; predatory thrips; *Tetranychus urticae***Author for correspondence:**Hajar Pakyari, Email: hajar.pakyari@iau.ac.ir**Abstract**

Cold storage effects on the female predatory thrips *Scolothrips longicornis* Priesner (Thysanoptera: Thripidae) fed on *Tetranychus urticae* Koch eggs for different time periods (5, 10, 20 and, 30 days) at 5 or 25 °C (control) on development and life table parameters were examined. Female adult duration and female total longevity were not influenced by the period of cold storage. The minimum mean female total longevity (28.1 ± 0.32 day) was observed in the 30-day treatment. The oviposition period decreased with increasing cold storage duration. All life table parameters of *S. longicornis* demonstrated a significant difference across treatments. Our results demonstrated that the female adult of *S. longicornis* could be maintained at 5 °C for 5-days with minimal reduction in the life-history process. This would be beneficial in the storage and mass production of *S. longicornis* in biocontrol programs involving *T. urticae*.

Introduction

The two-spotted spider mite, *Tetranychus urticae* Koch (Trombidiformes: Tetranychidae), has a worldwide distribution and damages more than 900 species of plants, including annual and perennial crops (Pakyari and Enkegaard, 2012). Control of two-spotted spider mite with synthetic pesticides is challenging because of its short development time, high fecundity, a habit of feeding on the lower leaf surface making the efficacy of acaricide applications problematic and rapid development of acaricide resistance (Pakyari and Enkegaard, 2015a). Biocontrol programs play a significant role in the population management of spider mite. Entomophagous control agents include a wide range of taxa such as acarophagous ladybird beetles (Coccinellidae), predatory anthocorids (Anthocoridae), predatory mites (Phytoseiidae) and predatory thrips (Thripidae) (Sabelis, 1985; Gotoh *et al.*, 2004; Trdan *et al.*, 2005; Kreiter *et al.*, 2020). The majority of the above-mentioned predators, feed on the most soft-bodied insects across several acarophagous but all species of the genera *Scolothrips* appear to be specialized spider mite predators (Pakyari and Enkegaard, 2015b).

Scolothrips longicornis Priesner (Thysanoptera: Thripidae) is widely distributed across the Middle East, North America, India and several European countries (Pakyari and McNeill, 2020). This species appears to be a specialized predator of *T. urticae* (Gilstrap and Oatman, 1976). *Scolothrips longicornis* is generally found in greenhouse and farm crops like cucumber, tomato, soybean and bean where two-spotted spider mite are present (Pakyari *et al.*, 2011a, 2011b).

Detailed studies of *S. longicornis* demographic parameters at different temperatures, functional response, prey stage preference and prey consumption have been measured (Pakyari *et al.*, 2009, 2011a, 2011b; Pakyari and McNeill, 2020), but no previous research has examined the effect of cold storage on species development duration and demographic parameters.

Cold storage of predatory insects for periods of 14 days and over can be an important tool for successful mass rearing and augmentative release. Storage at low temperatures also allows for the synchronized release of control agents into a crop when pest monitoring indicates potential economic loss to the crop, or in response to an undetected outbreak, where insecticide applications do not allow for the use of pesticides (e.g. in organic systems). In addition, the optimization and development of effective storage procedures at low-temperature for natural enemies can decrease the cost of biological control programs cost by extending the rearing period to synchronize with the biology of the target pest (Rezaei *et al.*, 2020).

Tolerance to cold storage and consequent effects on longevity and efficacy when released into the target environment varies amongst natural enemies (Rathee and Ram, 2018). Storage at low temperatures can have a negative effect on insect biology, reducing survival of both immatures and adults, sex ratio and fecundity (e.g. Rathee and Ram, 2018). When mass rearing natural enemies for inundative releases, it is also essential to choose the life stage that can tolerate low-temperature storage (Benelli *et al.*, 2018). In this respect, the

pupal and adult stages of predatory insects are considered to be the most appropriate stage for low-temperature storage (Abdel-Salam and Abdel-Baky, 2000; Zhang *et al.*, 2019).

For evaluating the quality of natural enemies, life table analysis provides a suitable measure of their efficiency and potential biological impacts on target hosts (Pakyari *et al.*, 2018; Rezaei *et al.*, 2020). The most critical parameters that have been utilized for the evaluation are the net reproductive rate (R_0), intrinsic rate of increase (r), finite rate of increase (λ), and mean generation time (T) (Carey, 2001). Furthermore, for the estimation of low-temperature storage of predators in biocontrol programs, the use of the cumulative fecundity curves of first-generation predators, net reproduction and emergence rate, has also been recommended (Rezaei *et al.*, 2020).

Nevertheless, previous research has not evaluated the influence of cold storage on the life table parameters of *S. longicornis*. To optimize mass rearing of *S. longicornis*, we determined the influence of low temperature storage at constant 5 °C for four time periods (5, 10, 20 and 30-days) on life tables of *S. longicornis*. We selected 5 °C since this temperature is above the low-temperature threshold of 3.96 °C estimated by Pakyari *et al.* (2011a). Another reason for selecting 5 °C was that several research studies have demonstrated that the appropriate temperature for storage of insect predators ranges from 1 to 10 °C (Abdel-Salam and Abdel-Baky, 2000; Sakaki *et al.*, 2019; Zhang *et al.*, 2019), and this provides a mid-point with which to assess effects on *S. longicornis* biological parameters. This contribution describes research to establish the optimal temperature for storage of *S. longicornis*, with a view of developing recommendations for commercial rearing operations in biocontrol programs targeting *T. urticae*.

Material and methods

Mite and thrips colony rearing

Two-spotted spider mite and *S. longicornis* were initially collected from a cucumber field in the Varamin Tehran province (35.3252° N, 51.6472° E and 920 m above sea level) in July 2016. These were maintained on excised leaves of cucumber which were put upside down on a piece of moist tissue paper in a plastic petri dish (15 cm diameter × 1 cm deep). Ventilation was provided by a 3 cm diameter hole drilled into the lid and covered by a thin nylon mesh secured with a water proof glue. Each petri dish was maintained in a controlled grow chamber at 25 ± 0.5 °C, 16L: 8D and 60 ± 10% r.h. To keep the *S. longicornis* culture, a single cucumber leaves infested with two-spotted spider mites was placed in a petri dish (18 cm diameter × 1 cm deep) as mentioned above. Every three days, adult predatory thrips are transferred to a new arena. The laboratory culture of *S. longicornis* was held for three months on two-spotted spider mite before been used for the experiments.

Host plant

Bean plants (*Phaseolus vulgaris* L. cv. Akhtar) were grown in a mixture of soil 65% and peat moss 35% in a glasshouse. Bean leaf disks (c. 3 cm in diameter) with minor veins were selected to provide the host plant for the mites. Each bean leaf was placed upside down on a piece of moist cotton inside a petri dish (6 cm in diameter × 1 cm deep). Ventilation was provided by a

1 cm diameter hole, drilled into the lid and covered by a thin nylon mesh secured at the margins with glue.

Cold storage bioassay

To estimate the influence of low-temperature storage on predatory thrips, twenty newly emerged predatory adults (1 day old) were maintained at a constant 5 °C for 5, 10, 20 and 30 days, at 16L: 8D, and 60 ± 10% r.h. As the control, one day old *S. longicornis* adults were maintained at 25 ± 0.5 °C, 16L: 8D and 60 ± 10% r.h. At the end of the cold storage treatment intervals thrips were transferred into an environment chamber set at 25 ± 0.5 °C, 16L: 8D and 60 ± 10% r.h. The subsequent assessment of biological performance was conducted under these conditions, with 20 mated adult females from each cold storage treatment transferred to Petri dishes. Females were placed with adult males in bean leaf disk for 48 h, to obtain mated females.

Females lay eggs singly in an incision made in the leaf disk with their ovipositor that distinguished as white elliptic floats on a leaf tissue that visualized under binocular (×20). For each treatment, 30 (24 h old) *S. longicornis* eggs were held in an environmental chamber. Newly hatched larvae were provided daily with sufficient eggs of *T. urticae* (about 100 spider mite eggs); the number of offered preys was greater than the utilization capacity (Pakyari and Enkegaard, 2012). The bean leaf disks were replaced every three days until death. One male and female (1 day old) were placed onto the leaf disks to allow for mating. Thereafter, they were checked daily to determine the initiation of egg production.

Life table analysis

Longevity, survival rate, development rate and daily fecundity of *S. longicornis* females were calculated utilizing the age-stage, two-sex life table method and theory (Chi and Liu, 1985; Chi, 1988) and analyzed using the TWOSEX-MSChart software (Chi, 2020). The age-specific oviposition rate (m_x), the age-specific survival rate (l_x), the age-stage-specific survival rate (s_{xj}) (x = age, j = stage) and the age-stage-specific fecundity (f_{xj}) along with the population parameters including the net reproductive rate (R_0), intrinsic rate of increase (r), finite rate of increase (λ), and mean generation time (T), were analyzed according to Chi and Su (2006). The adult preoviposition period (APOP) and total preoviposition period (TPOP) were also calculated. The mean and standard error of life table parameters were estimated with the bootstrap method with 10,000 resampling to get a reliable estimation (Efron and Tibshirani, 1994). To establish differences between cold storage treatments, the paired bootstrap test was then performed.

Result

Development, longevity, and reproduction by *S. longicornis*

Cold storage did not significantly influence the female preadult duration but significantly affected the male preadult duration (table 1). Female adult duration and female total longevity were not influenced by the cold storage treatment durations but all were significantly different from the control. The mean minimum and maximum female total longevity were recorded in the 30-day low-temperature storage (28.1 ± 0.32 d), and the control (33.7 ± 1.03 d) treatments, respectively. The oviposition period decreased

Table 1. Mean (\pm SE) development time (days) of immature stages, mean longevity, fecundity, total preoviposition period (TPOP), adult preoviposition period (APOP), and oviposition periods of *Scolothrips longicornis* reared on eggs of *Tetranychus urticae* at 5 °C for different cold storage intervals

Stage	n	Control	n	5-day	n	10-day	n	20-day	n	30-day	$F_{4, 92}$	P
Female preadult duration (d)	20	13.15 \pm 0.31a	17	12.47 \pm 0.45 a	19	13.25 \pm 0.46a	21	13.90 \pm 0.31a	20	13.85 \pm 0.26a	$F_{4, 92} = 2.52$	$P = 0.046$
Male preadult duration (d)	9	12.44 \pm 0.63ab	7	11.57 \pm 0.57b	7	12.71 \pm 0.75ab	5	14.40 \pm 0.60a	6	14.50 \pm 0.50a	$F_{4, 29} = 3.70$	$P = 0.015$
Female adult duration (d)	20	20.50 \pm 0.94a	17	15.76 \pm 0.72b	19	14.95 \pm 0.61b	21	14.52 \pm 0.54b	20	14.25 \pm 0.28b	$F_{4, 92} = 16.33$	$P < 0.001$
Male adult duration (d)	9	17.44 \pm 1.39a	7	15.86 \pm 1.26ab	7	14.71 \pm 1.04ab	5	13.2 \pm 1.2ab	6	12.17 \pm 0.01b	$F_{4, 29} = 2.90$	$P = 0.039$
Female total longevity (d)	20	33.65 \pm 1.03a	17	28.24 \pm 0.98b	19	28.21 \pm 0.89 b	21	28.43 \pm 0.67b	20	28.10 \pm 0.32b	$F_{4, 92} = 9.18$	$P < 0.001$
Male total longevity (d)	9	29.89 \pm 1.45a	7	27.43 \pm 1.17a	7	27.43 \pm 0.87a	5	27.60 \pm 1.36a	6	26.67 \pm 1.23a	$F_{4, 29} = 1.08$	$P = 0.384$
Oviposition period (d)	20	13.90 \pm 0.76a	17	10.18 \pm 0.56b	19	8.95 \pm 0.51b	21	8.62 \pm 0.42b	20	8.45 \pm 0.34b	$F_{4, 92} = 18.55$	$P < 0.001$
APOP (d)	20	1.60 \pm 0.15a	17	1.53 \pm 0.12a	19	1.89 \pm 0.17 a	21	2.10 \pm 0.18a	20	2.15 \pm 0.20a	$F_{4, 92} = 2.73$	$P = 0.034$
TPOP (d)	20	14.75 \pm 0.31ab	17	14 \pm 0.47b	19	15.16 \pm 0.51ab	21	16 \pm 0.37a	20	16 \pm 0.35a	$F_{4, 92} = 4.37$	$P = 0.003$
Fecundity (offspring/female)	20	52.75 \pm 3.05a	17	25.06 \pm 1.86b	19	17.95 \pm 1.45c	21	15.05 \pm 0.93d	20	13.95 \pm 0.87e	$F_{4, 92} = 18.55$	$P < 0.001$
$N_f/N_{f, ratio}$	30	0.67 \pm 0.09b	30	0.57 \pm 0.09d	30	0.63 \pm 0.09c	30	0.70 \pm 0.10a	30	0.67 \pm 0.09ab	$F_{4, 92} = 41.70$	$P < 0.001$

N_f : Number of individuals survived to the adult stage. Values followed by small different letters within the same row are significantly different (paired bootstrap test, $P < 0.05$).

with increasing duration of cold storage with all these treatments significantly different from the control (table 1). The sex ratio of offspring was affected by the period of cold storage with the maximum was monitored in the 20-day cold storage and minimum was observed in the 5-day cold storage.

Age-stage, two-sex life table

The age-stage specific survival curves indicate the likelihood that a newly laid egg surviving to stage j and age x . The s_{xj} curves for *S. longicornis* at each duration of cold storage overlapped, indicating a difference in development rates among individuals. The age-specific survival rate (l_x), of *S. longicornis* adults were 0.97 for the control, 0.87 for 5-day cold storage duration and 0.93 for the 10, 20 and 30-day cold storage durations (fig. 1). The age-specific survival rate (l_x), age-stage-specific fecundity (f_{x5} , adult is the five-life stage), age-specific oviposition rate (m_x), and age-specific maternity ($l_x m_x$) for each treatment are presented in fig. 2. The oviposition period decreased with increasing duration of cold storage. *S. longicornis* took more time to achieve their peak m_x at the 5-day cold storage treatment (fig. 2).

Life table parameters for *S. longicornis*

The life table parameters for the four cold storage and control treatments are shown in table 2. For all life table parameters, there were significant differences across treatments. The intrinsic rate of increase (r_m , d^{-1}) was lowest for the 30-day cold storage treatment (0.106 ± 0.007) and the highest for the control (0.169 ± 0.008). Mean generation time (T) was the shortest for the 5-day cold storage treatment (19.36 ± 0.58) and the longest for the 20-day cold storage treatment (21.25 ± 0.39).

Discussion

Commercial production of beneficial insects requires periodic rearing followed by the ability to maintain the predators produced in a healthy state, especially when used in augmentative biocontrol release programs (Rathee and Ram, 2018). Storage of natural enemies at cold storage is a simple method for maintaining a large number of the natural enemies dormant for short- and long-term and talking them to appropriate conditions for reproduction and to control pests when needed (Hodek et al., 1973). Age-stage two-sex life tables were utilized to determine the reproductive and developmental biology of *S. longicornis* following low-temperature storage of the adult at a different duration. Our study demonstrated that overall, maintaining *S. longicornis* adults at 5 °C was detrimental to the longevity, fecundity, oviposition period, and life table parameters compared to the control treatment. Decreasing temperature from the optimum will commonly decrease the rate of metabolic, oxygen consumption, developmental rates and activity of beneficial insects to stretch their lives, which is the foundation for utilizing cold storage (Liu and Ridsdill-Smith, 2000). The effect of cold storage on biological parameters of predatory insects has been examined in several studies including, *Coccinella septempunctata* and *Adalia bipunctata* (Col.: Coccinellidae) (Hamulainen and Markkula, 1977), *Concinella undecimpunctata* (Col.: Coccinellidae) (Abdel-Salam and Abdel-Baky, 2000), *Cryptolaemus montrouzieri* (Col.: Coccinellidae) (Ozgoke et al., 2006), *Orius insidiosus* (Hem.: Anthocoridae) (Bueno et al., 2014), *Rodolia cardinalis* (Col.: Coccinellidae) (Abdel-Baky et al., 2015), *Podisus nigrispinus*

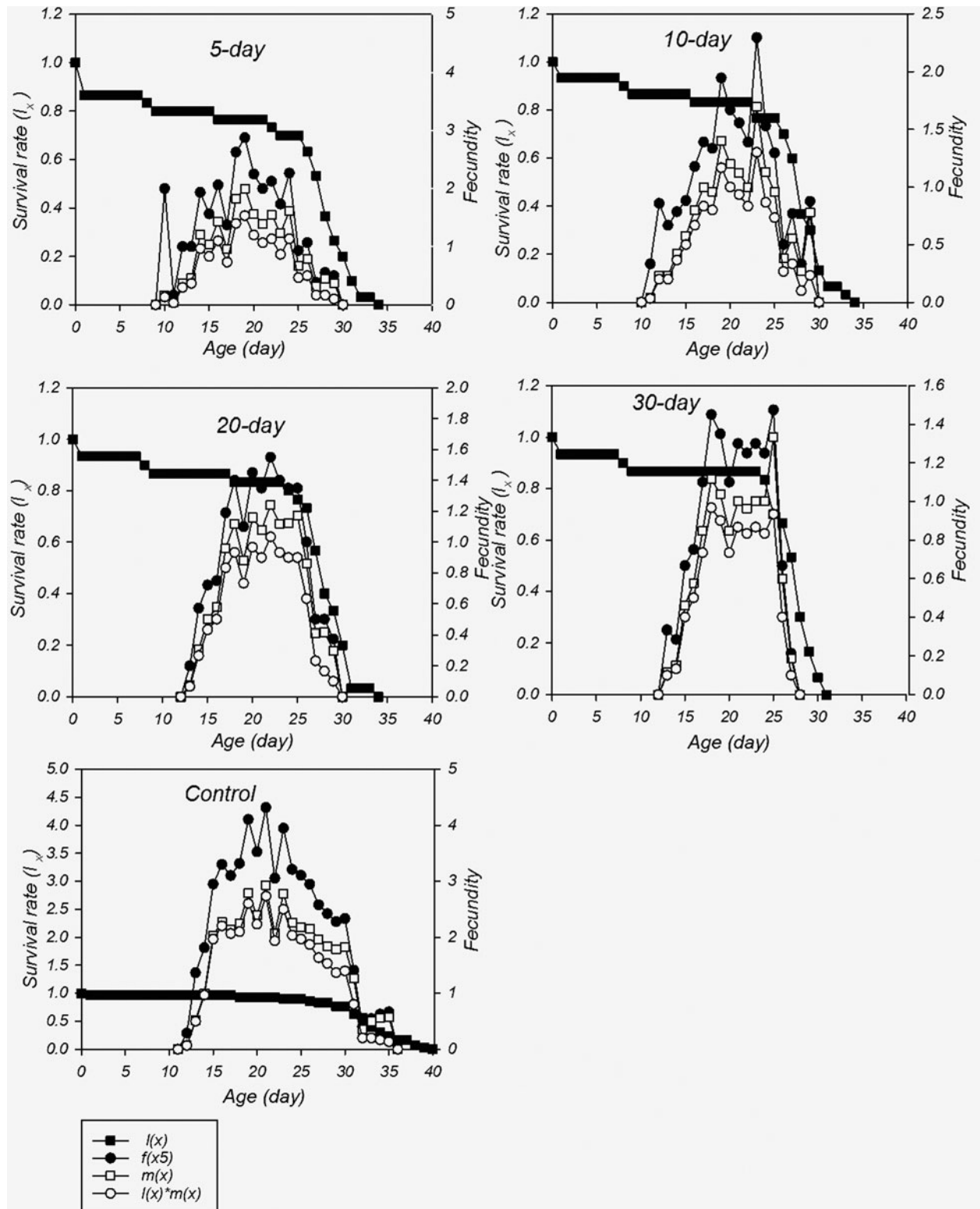


Figure 1. *Scolothrips longicornis* survival rate (s_{xj}) for eggs, larvae, prepupa, pupae and adults (both female and male) reared on *Tetranychus urticae* eggs following storage at 5 °C for 5, 10, 20 and 30 days compared to the control treatment (25 ± 0.5 °C). The s_{xj} depicts the probability that an individual can survive to age x and stage j . The variable developmental rates among individuals are depicted as the overlaps between different stages during developmental periods.

(Hem.: Pentatomidae) (Costa *et al.*, 2016) and *Rhyzobius lophantae* (Col.: Coccinellidae) (Senal *et al.*, 2017). But this study is the first experiment about the influence of cold storage for various storage durations on *S. longicornis*.

Our results demonstrate that the development time of *S. longicornis* females not significantly different with extending the storage period at 5 °C. But in males, development time increases with increasing the storage period from 10 to 30-day. It also suggests

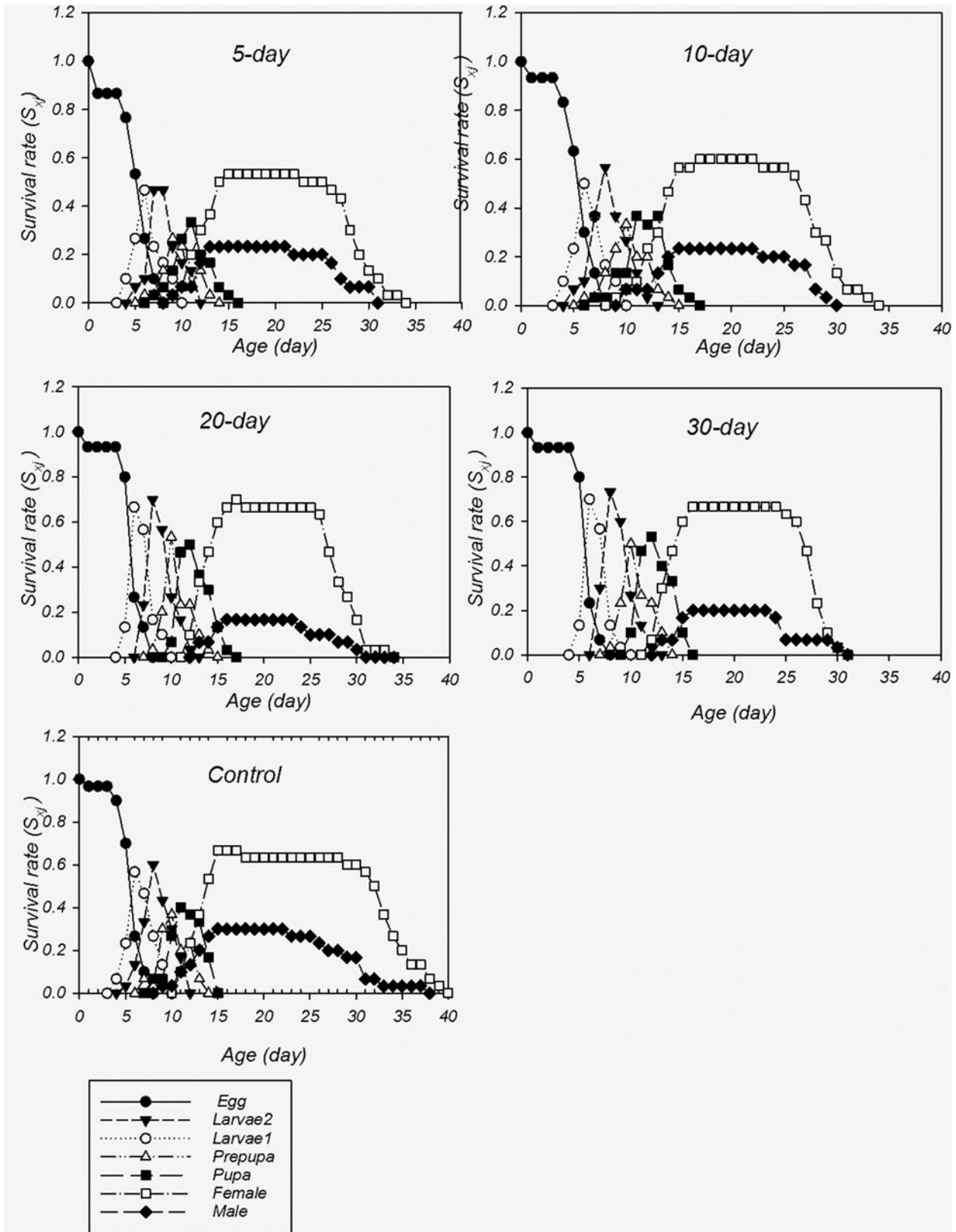


Figure 2. Age-specific survival rate (l_x), oviposition rate (m_x), maternity ($l_x m_x$), and age-stage-specific fecundity (f_{x5}) of *Scolothrips longicornis* adults reared on *Tetranychus urticae* eggs following storage at 5 °C for 5, 10, 20 and 30 days, compared to the control treatment (25 ± 0.5 °C). Note different Y-scale for the control treatment.

Table 2. Mean (\pm SE) intrinsic rate of natural increase (r_m), finite rate of increase (λ), net reproductive rate (R_0), and mean generation time (T) of *Scolothrips longicornis* reared on eggs of *Tetranychus urticae* following storage at 5 °C for different intervals compared to the control (25 \pm 0.5 °C)

	Control	5-day	10-day	20-day	30-day	
r_m (d^{-1})	0.169 \pm 0.008a	0.138 \pm 0.010b	0.119 \pm 0.009c	0.111 \pm 0.007d	0.106 \pm 0.007e	$F_{4, 92} = 994.99, P < 0.001$
λ (d^{-1})	1.184 \pm 0.009a	1.147 \pm 0.012b	1.126 \pm 0.010c	1.117 \pm 0.007d	1.112 \pm 0.008e	$F_{4, 92} = 1012.54, P < 0.001$
R_0 (offspring)	35.17 \pm 4.96a	14.20 \pm 2.59b	11.37 \pm 1.92c	10.53 \pm 1.41c	9.30 \pm 1.30d	$F_{4, 92} = 1542.26, P < 0.001$
T (d)	21.11 \pm 0.39b	19.36 \pm 0.58d	20.51 \pm 0.53c	21.25 \pm 0.39a	21.02 \pm 0.37b	$F_{4, 92} = 336.89, P < 0.001$

Values followed by small different letters within the same column are significantly different (paired bootstrap test, $P < 0$).

that a greater female storage tolerance might be relevant to their greater body size (e.g. Queiroz *et al.*, 2016). Female and male adult duration decreased with increasing the cold storage period.

The female proportion in the cohort is also investigated as an important factor in the augmentative release of biological control programs (Rezaie *et al.*, 2020). In this research, the sex ratio of *S. longicornis* was significantly influenced by different duration of low-temperature storage. The proportion of females became more female-biased with extending storage duration and included more females when stored longer. In 5-day cold storage, the least effect on the proportion of females and this storage duration better tolerance to low temperatures compared to other treatments.

Oviposition period was also found to decrease with an increase in storage duration, and agrees with other research on parasitoid and predatory insects (e.g. Rundle *et al.*, 2004; Silva *et al.*, 2013; Rathee and Ram, 2014). One reason for this effect can be the storage relation between the adult longevity and fat reserves rate (Rathee and Ram, 2014; Kidane *et al.*, 2015). Additionally, there was no significant difference in the preoviposition period of adults (APOP) after a different cold storage period.

Mean total fecundity of *S. longicornis* ranged from 52.8 eggs per female (control treatment) to 13.9 (30-day treatment). Total fecundity decreased with increasing duration of cold storage. Low-temperature storage can either cause reproductive organ deformity or egg maturation delay (Colinet and Boivin, 2011). The present result agrees with other studies conducted on parasitoid and predatory insects (Rathee and Ram, 2014).

An insect's demographic parameters can depend on different factors, involving conditions of rearing (Pakyari *et al.*, 2011a). Few research studies have determined the demographic parameters of beneficial insect's subject to cold storage prior to use in biocontrol programs (Ismail *et al.*, 2014; Rezaei *et al.*, 2020). In this research, all population parameters varied the four cold storage intervals. The calculated value of life table parameters of the intrinsic rate of increase (r), net reproductive rate (R_0) and finite rate of increase (λ), were decreased by increasing cold storage duration. It is well known that even a little decrease in the intrinsic rate of increase can cause big changes in the size of the population (Ozgoke *et al.*, 2006). After 5 days of storage, the intrinsic rate of increase (0.169 at 25 °C) was lower than those demonstrated by Pakyari *et al.* (2011b) (0.201 at 26 °C). The differences between their results with the results presented here may be related to utilizing the traditional female-centric life table analysis. In traditional life table analysis, the male population component is ignored and based on female age-specific parameters (Pakyari and McNeill, 2020). Since the sex ratio plays an essential role in population growth and life table rate is usually concentrated on parameters of female, we recommend that the age-stage, two-sex life table could be utilized in future research.

In conclusion, achieving an optimal cold storage temperature and duration depends on the field implementation of the low temperature stored parasitoid and predator insects. But, there are very little researches that have determined post-storage efficiency by open crop system and greenhouse experiments. Creation in low-temperature storage in near future can reduce the cost of mass rearing of beneficial insects, therefore making biocontrol more economical, ready to implement and easier. These results indicate that *S. longicornis* adults may be stored at 5 °C for five days without producing significant effects on the population parameters, especially fecundity that affect their efficiency as a biocontrol agent targeting *T. urticae*.

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