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Article

## Prolonged coldness on eggs reduces immature survival and reproductive fitness in *Tetranychus urticae* (Acari: Tetranychidae)

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### Abstract

*Tetranychus urticae* Koch (Arachnida: Acari: Tetranychidae) is an economically important agricultural and horticultural pest around the world. The present study investigated the prolonged exposure of eggs (i.e., 0, 24, 48, 72, and 96 hours) to low temperature (i.e., 5 °C) affecting the egg hatching, and subsequent immature development and survival as well as the reproductive fitness of resultant females. Our results show that chilling had no significant effect on egg hatching rate, but significantly shortened egg development. It was found that prolonged chilling significantly decreased the survival of immature stages. Prolonged chilling also significantly delayed the development of males and thus adult emergence, but had not significantly effect on female development and survival. Results further indicate that the negative effect of lengthy chilling extended to the resultant females resulting in the significantly shorter longevity and lower fecundity. It is interesting that the resultant females might benefit from parental egg chilling, i.e., they started oviposition significantly early and produced significantly higher female-biased sex ratio. The implications from the results of this study in forecasting spring populations and outbreak of this pest in the growing season were discussed.

**Key words:** Spider mite, chilling duration, development, spring population.

### Introduction

The two-spotted spider mite, *Tetranychus urticae*, is one of the most economically important agricultural and horticultural pests with a global distribution, which feeds on a wide range of host plant species (Zhang 2003; Xie *et al.* 2006; Chhillar *et al.* 2007; van Leeuwen *et al.* 2010). The life cycle of *T. urticae* includes egg, larva, protonymph, deutonymph and adult stages. The mites suck plant sap via piercing-sucking mouthparts, causing damage to the leaves, buds and immature stems of host plants (Meyer & Craemer 1999).

Spider mites are arrhenotokous and haplodiploid species, i.e., unmated females produce only unfertilized haploid eggs which develop to males, and mated females lay both unfertilized haploid eggs and fertilized diploid eggs that develop to male and female offspring, respectively (Macke *et al.* 2011). Bounfour & Tanigoshi (2001) reported that *T. urticae* requires about 7.4 days to complete development at 30 °C with more than 120 eggs produced. Thus the properties of rapid developmental rate and high reproductive potential associated with the arrhenotokous parthenogenesis in *T. urticae* will allow the quick build-up of field populations under the warm climate conditions.

*Tetranychus urticae* is an invasive species and first recorded in 1983 in Beijing, China (Dong *et al.* 1987). The details of seasonal phenology of *T. urticae* in fields are not clear in China. Damage in

apple orchards was first observed in Changli County, Hebei Province, and Lanzhou City and Tianshui City, Gansu Province during early 1990s (Piao *et al.* 1993). Three pest mite species, the *T. urticae*, *Amphitetranychus viennensis* Zacher and *Panonychus ulmi* (Koch), usually simultaneously occur in orchards, but *T. urticae* gradually became dominant and eventually replaced the other two as the key species during mid-late 1990s (Yu *et al.* 1998). In mid-1990s, outbreaks of *T. urticae* and serious damage in the apple, pear and peach orchards have been reported in warm spring in Beijing and provinces of Hebei, Shandong, Liaoning, Shanxi and Gansu (Zhou *et al.* 2003). However, except that in greenhouses, the field populations of *T. urticae* sharply decrease since 2003, and now mites are rarely found in North and Northeast China (L. Yu, person. comment). This may be caused by the low temperatures during early spring (Anon 1993–2013). Raworth (2007) found that in southwestern British Columbia, Canada, many herbivorous and predaceous mites that reactivated in early February, 2006, died after a long period (9 days) of frost during mid-late February.

It is known that *T. urticae* overwinter as fertilized female adults (Cone *et al.* 1986). Raworth (2007) predicted that in southwestern British Columbia, Canada, the probability of *T. urticae* adults developing from winter diapause to initial oviposition is linearly related to temperature above 9.4 °C, and oviposition most likely occur during early and mid-March. It is reported that in China, the overwintered *T. urticae* adults become active when the mean temperature reaches 3 °C (Sun 2000) and start laying eggs when the mean temperature is above 6–7 °C (Cheng 2012) which is lower than that estimated by Raworth (2007). According to the local climate conditions (i.e., Anon 1993–2013), it can be assumed that in Changli County, *T. urticae* adults emerge during early to mid-March and oviposit in late March during which period the mean temperature is higher than 7 °C.

Unfortunately, the daily temperature usually fluctuates intensely in nature, i.e., it is frequently less than 5 °C and lasts successively for up to 6 days (Anon 1993–2013). Abukhashim & Luff (1997) revealed that chilling (0, -5 and -10 °C) and prolonged chilling from 0 to 16 hours have significant negative effects on the survival of each life stages and reproductive output of female adults, and eggs are more sensitive to the hard environmental conditions than other life stages. In Changli County, the mean temperature of subzero rarely occurs during late March (Anon 1993–2013); thus the immature and adult stages are unlikely to experience extremely low temperature in the fields. Furthermore, Abukhashim & Luff (1997) detected the reproduction of chilled females but did not follow the reproductive fitness of resultant females whose early immature stage(s) had experienced the low temperatures. The fitness of those resultant females may be impacted by the prolonged chilling duration that they have experienced during parental generation (e.g., Jones & Kunz 1998; Chen *et al.* 2008; Mockett & Matsumoto 2014). Cone *et al.* (1986) have also preliminarily observed the reproduction and sex allocation of overwintered *T. urticae* females at constant temperature (18–20 °C), but they did not investigate the possibility of low temperature, for example during spring, affecting the survival and development of egg and immature stages. These may make it difficult to apply the data of Cone *et al.* (1986) and Abukhashim & Luff (1997) to forecast the seasonal population dynamics of *T. urticae* in the fields.

Basing on the above known knowledge of *T. urticae*, we carried out this preliminary study to test whether and how chilling duration of *T. urticae* eggs at 5 °C influenced egg hatching rate, subsequent immature development and survival, and reproductive fitness of resultant females. Information of this study associated with that of local climate is vital to the spring population forecast, pest risk analysis and timing of management of *T. urticae*.

## Materials and Methods

### *Mites*

Because of the difficulty of collecting overwintering adults or eggs during early spring (March), the breeding colony of *T. urticae* started from specimens ( $\approx 500$  individuals of mixed life stages) collected from cherry (*Prunus cerasus* L.) trees late April, 2014, in the courtyard of Changli Research Institute of Pomology, Changli County (39°43'N, 119°10'E), Hebei Province, China. Mites were reared on peach (*Amygdalus persica* L.) leaves ( $\approx 3$  cm in diameter) placed upside down on wet cotton in Petri dishes (5.0 cm diameter  $\times$  2.0 cm height). The mite colony was maintained in a plant growth chambers (LRH-250GS, China) with controlled conditions of  $25 \pm 1$  °C, 70% HR and 14:10 h (Light:Dark). Mites were reared for three generations before the experiment started.

### *Development and survival of eggs and immature stages*

To obtain eggs for experiment, 30 adult females from the breeding colony were inoculated on a peach leaf in a Petri dish. After 12 hours, the females were removed, and eggs were counted under a binocular dissecting microscope (Leica EZ4, Germany) with 30–40 eggs remained on a leaf disk. Eggs were transferred to the above mentioned plant growth chamber. To avoid thermal shock when eggs were transferred to the low temperature, we decreased the temperature gradually from 25 °C to 5 °C with a reduction rate of 0.1 °C/minute. Eggs was maintained at 5 °C for 0 (control), 24, 48, 72 or 96 h under dark condition. After egg chilling for a desired duration, the temperature increased gradually from 5 °C to 25 °C with an increasing rate of 0.1 °C/minute. Eggs were finally maintained in the plant growth chambers with the same environmental conditions as that for the breeding colony. Eggs were then observed every 12 hours for hatching and the number of eggs hatched was recorded for each leaf. The developmental duration of eggs excluding the chilling duration was also recorded. There were 150, 154, 187, 114 and 150 eggs for the above treatments, respectively.

After egg hatching, all larvae were individually collected from the leaves and relocated on a new peach leaf ( $\approx 2$  cm in diameter) in a Petri dish using a fine paintbrush. The developmental duration and number of larvae, protonymphs and deutonymphs that successfully developed to next stage were recorded, and the developmental duration from egg hatching to adult emergence was also calculated. Newly emerged adults were sexed.

### *Reproductive fitness of resultant female offspring*

After adult emerged, a female and a male were paired on a peach leaf in a Petri dish under the above mentioned environmental conditions. After 24 h, the female was transferred to a new leaf by the fine paintbrush. This process was repeated until the mother died. The male was separated from the female and maintained on a new leaf until death. Daily number of eggs laid by the mother was counted under the dissecting microscope and egg hatching was recorded. Hatched larvae were allowed to feed and develop on the same leaf which was replaced with a new one every four days. For each mother, the pre-oviposition period (i.e., time period between adult emergence and oviposition) and fecundity (i.e., total number of eggs laid) were recorded, and the survival rate from egg to adult emergence (i.e., total number of adults emerged/total number of eggs laid) and secondly offspring sex ratio (female offspring%) were calculated. The longevity of both males and females was also recorded. There were 21, 38, 52, 34 and 15 females tested for treatments of 0, 24, 48, 72 and 96 h, respectively.

### *Statistical analysis*

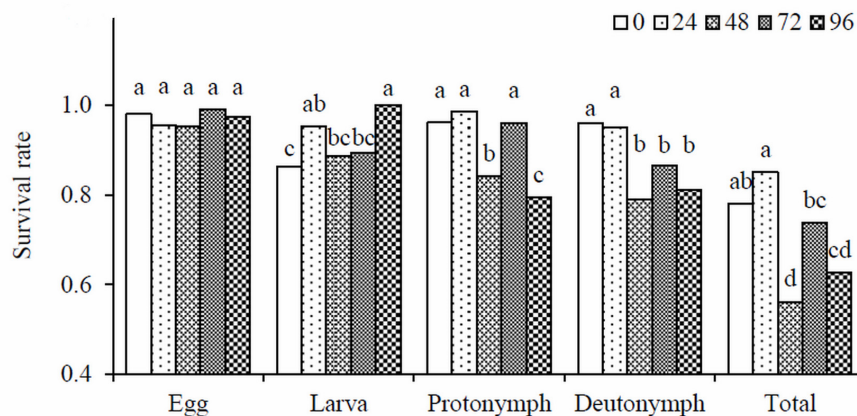
A likelihood rate test (LR, GENMOD Procedure) was used to compare the difference in egg hatching rate and survival rate of immature stages between treatments. A goodness-of-fit test (Shapiro-Wilk test, UNIVARIATE Procedure) was used to test the distribution of other data before analysis. Data

of fecundity and egg hatching rate of resultant females were analysed using an analysis of variance (ANOVA, GLM Procedure), followed by a Tukey's studentised range test. However, data on female fecundity and longevity were square-root transformed and that on egg hatching rate of resultant females were arcsine square-root transformed before analysis. Data on the development of immature stages, female pre-oviposition period, proportion of female offspring and male longevity were not normally distributed even after transformation. Therefore, these data were analyzed using a nonparametric ANOVA (GLM Procedure) followed by Bonferroni (Dunn) t Tests for multiple comparison. The survival of resultant male and female adults was analyzed using Lifetest (LIFETEST Procedure). All analyses were done using SAS (SAS 9.4, SAS Institute Inc., NC, USA).

## Results

### *Development and survival of eggs and immature stages*

As shown in Figure 1, chilling had no significant effect on egg hatching rate (> 95%) (LR:  $x_4^2 = 5.88$ ,  $P = 0.2082$ ). However, survival rate of larvae, protonymphs and deutonymphs varied significantly between treatments, i.e., it was significantly higher when egg chilling lasted for 96 h than for only 0, 48, and 72 h for larvae (LR:  $x_4^2 = 28.32$ ,  $P < 0.0001$ ), significantly higher for treatments of 0, 24 and 72 h than for that of 48 and 96 h for protonymphs (LR:  $x_4^2 = 48.52$ ,  $P < 0.0001$ ), and significantly higher for treatments of 0 and 24 h than for other treatments for deutonymphs (LR:  $x_4^2 = 30.59$ ,  $P < 0.0001$ ) (Figure 1). The final survival rate was significantly higher when eggs were chilled for 24 h than for 48, 72 and 96 h (LR:  $x_4^2 = 44.19$ ,  $P < 0.0001$ ) (Figure 1).



**FIGURE 1.** Chilling duration (hours) affecting egg hatching rate and survival rate of immature stages of *T. urticae*. For each category, columns with the same letters are not significantly different ( $P > 0.05$ ).

Compared with the control, eggs developed significantly faster after chilling (ANOVA:  $F_{4,155} = 8.78$ ,  $P < 0.0001$ ; Figure 2) indicating that eggs could still develop under chilling conditions.

For males, larvae developed significantly slower when eggs were chilled for 96 h than for 24 h and control (ANOVA:  $F_{4,91} = 3.67$ ,  $P = 0.0081$ ); while protonymphs developed significantly slower when eggs were chilled for 24 h than for 48 and 72 h (ANOVA:  $F_{4,91} = 3.94$ ,  $P = 0.0054$ ) (Table 1). However, the developmental duration of deutonymphs was significantly longer for treatments of 24 and 72 h chilling than that of 48 h chilling and control (ANOVA:  $F_{4,91} = 10.70$ ,  $P < 0.0001$ ) (Table 1). The total developmental duration from egg hatching to adult emergence was significantly longer for 96 h and 72 h and 24 h chilling than for 48 h chilling and control (ANOVA:  $F_{4,91} = 5.02$ ,  $P = 0.0011$ ) (Table 1).

For females, egg chilling did not significantly affect the development of larvae and protonymphs (ANOVA:  $F_{4,155} = 2.23$  and  $1.16$  respectively for larvae and Protonymphs,  $P > 0.05$ ); while deutonymphs developed significantly faster if eggs were chilled for 48 h than that for 24 h (ANOVA:  $F_{4,155} = 6.23$ ,  $P = 0.0001$ ) (Table 1). However, chilling had no significant effect on the total developmental duration from egg hatching to adult emergence (ANOVA:  $F_{4,155} = 1.08$ ,  $P = 0.3686$ ) (Table 1).

Results indicate that males developed from larva to adult stage significantly faster than did females for treatments of control, and 24 and 48 h chilling (ANOVA:  $F_{1,38} = 47.29$ ,  $P < 0.0001$  for control;  $F_{1,46} = 16.12$ ,  $P = 0.0002$  for 24 h chilling;  $F_{1,73} = 5.74$ ,  $P = 0.0192$  for 48 h chilling) with no significant difference detected between sexes for treatments of 72 and 96 h chilling (ANOVA:  $F_{1,48} = 0.69$ ,  $P = 0.4099$  for 72 h chilling;  $F_{1,23} = 0.13$ ,  $P = 0.7197$  for 96 h chilling) (Table 1).

**TABLE 1.** Egg chilling duration (h) at 5 °C affecting developmental duration (days) of immature stage of *T. urticae* at 25 °C.

Duration	Larva	Protonymph	Deutonymph	Larva–adult
Male				
0	2.18 ± 0.09 b	1.89 ± 0.06 ab	2.00 ± 0.04 b	6.07 ± 0.11 c
24	2.25 ± 0.06 b	2.09 ± 0.05 a	2.34 ± 0.08 a	6.69 ± 0.11 ab
48	2.55 ± 0.13 ab	1.77 ± 0.07 b	1.83 ± 0.05 b	6.16 ± 0.18 bc
72	2.47 ± 0.23 ab	1.75 ± 0.11 b	2.41 ± 0.10 a	6.63 ± 0.29 ab
96	3.00 ± 0.37 a	1.95 ± 0.23 ab	2.10 ± 0.27 ab	7.05 ± 0.67 a
Female				
0	2.74 ± 0.09 a	1.98 ± 0.07 a	2.35 ± 0.09 ab	7.06 ± 0.09 a
24	2.42 ± 0.06 a	2.22 ± 0.06 a	2.66 ± 0.08 a	7.30 ± 0.10 a
48	2.79 ± 0.12 a	2.16 ± 0.13 a	1.99 ± 0.13 b	6.95 ± 0.18 a
72	2.43 ± 0.08 a	2.09 ± 0.09 a	2.37 ± 0.12 ab	6.89 ± 0.13 a
96	2.43 ± 0.17 a	2.11 ± 0.11 a	2.51 ± 0.18 ab	7.06 ± 0.17 a

For each sex, means (± SE) with the same letters in each column are not significant different ( $P > 0.05$ ).

**TABLE 2.** Egg chilling duration (h) at 5 °C affecting reproduction fitness of *T. urticae* females at 25 °C.

Duration	Pre-oviposition period (days)	Fecundity (eggs)	Female offspring (%)
0	2.52 ± 0.18 a	48.43 ± 5.59 a	61.11 ± 4.50 b
24	2.49 ± 0.14 a	50.13 ± 3.80 a	60.90 ± 3.50 b
48	1.64 ± 0.11 b	33.73 ± 2.04 b	60.68 ± 2.73 b
72	1.75 ± 0.13 b	30.76 ± 1.95 bc	81.52 ± 1.82 a
96	1.90 ± 0.20 b	20.00 ± 3.08 c	74.86 ± 5.14 a

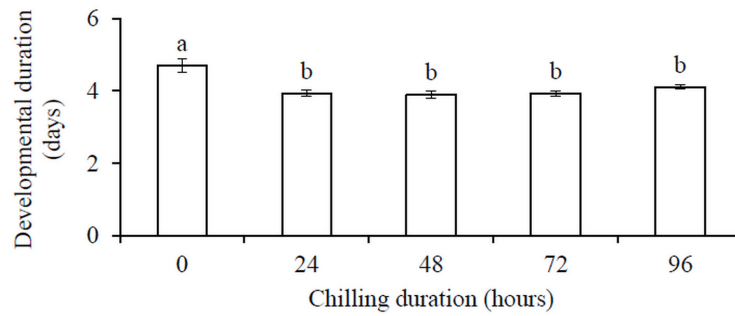
Means (± SE) with the same letters in each column are not significant different ( $P > 0.05$ ).

#### *Reproductive fitness of resultant female offspring*

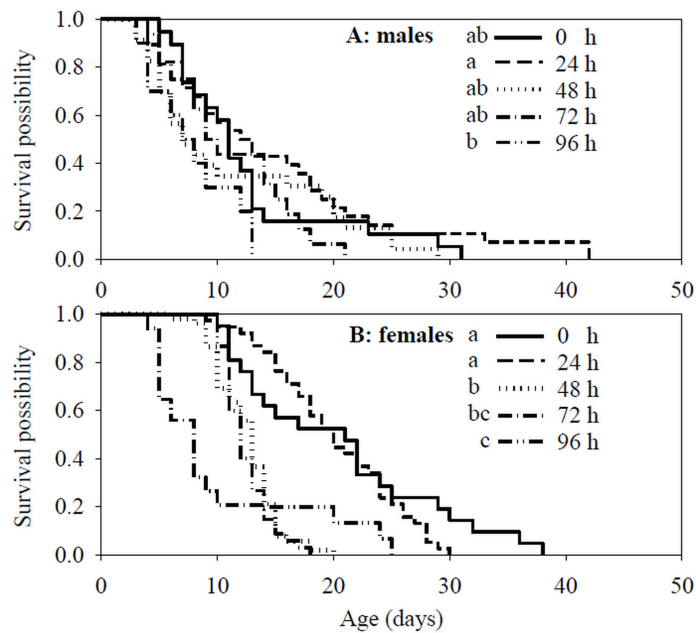
As shown in Table 2, preoviposition period of females was not significantly different between control and 24 h expose, while it became significantly shorter when the chilling duration extended to ≥ 48 h (ANOVA:  $F_{4,155} = 12.64$ ,  $P < 0.0001$ ). The life time fecundity significantly decreased if the chilling duration increased to ≥ 48 h (ANOVA:  $F_{4,155} = 11.92$ ,  $P < 0.0001$ ); however, the proportion of female offspring was significantly higher at the longer chilling durations of 72 and 96 h than that at shorter ones (i.e., 24 and 48 h) and control (ANOVA:  $F_{4,155} = 11.85$ ,  $P < 0.0001$ ) (Table 2). The

survival rate of immature stages varied from 56.9% to 65.2% which was not significantly different between treatments (ANOVA:  $F_{4,155} = 1.15$ ,  $P = 0.3348$ ).

Egg chilling had significant negative effect on adult longevity, i.e., male longevity was significantly longer when eggs were chilled for 24 h than 96 h (Lifetest:  $x_4^2 = 9.83$ ,  $P = 0.0434$ ) (Figure 3A), and compared with control and 24 h chilling, females lived significantly shorter when the egg chilling duration prolonged to 48, 72 and 96 h (Lifetest:  $x_4^2 = 55.82$ ,  $P < 0.0001$ ) (Figure 3B).



**FIGURE 2.** Chilling affecting development of *T. urticae* eggs. Columns (mean ± SE) with the same letters are not significantly different ( $P > 0.05$ ).



**FIGURE 3.** Egg chilling affecting survival of male (A) and female adults (B) of *T. urticae*. For each sex, lines (treatments) with the same letters are not significantly different ( $P > 0.05$ ).

## Discussion

Studies of low temperature affecting the development, survival and reproduction of arthropod species usually focus on the mechanisms of diapause induction and termination (seasonal long-term cycles, which can be predicted) (Tauber & Tauber 1976; Tauber *et al.* 1986). It is well known that diapause-terminated individuals are most likely to experience cold temperature of various durations



during early spring (aseasonal short-term events that can not be predicted) which may influence their development, survival and reproduction, but receiving less attention (see Tauber *et al.* 1986). Only a few studies follow the reproductive fitness of resultant females after insects have passed several live stages under the cold conditions (e.g., Jones & Kunz 1998; Chen *et al.* 2008, 2011; Mockett & Matsumoto 2014). Our study investigated the effect of prolonged chilling duration of *T. urticae* eggs at 5 °C on egg hatching, immature development and survival as well as reproductive fitness of resultant females. Although eggs used for experiment were not collected directly from the overwintering population, results from this study have implications in forecasting the spring population dynamics.

Abukhashim & Luff's (1997) reported that when temperature is  $\leq 0$  °C prolonged egg chilling from one to 16 hours significantly reduced the egg hatching rate. However, our results show that variation of chilling duration at 5 °C did not significantly affect egg hatching rate (Figure 1). This result has three implications. First, *T. urticae* eggs are more tolerant at 5 °C than at subzero temperatures, because injury may have occurred temperature  $\leq 0$  °C (Abukhashim & Luff 1997). It has been reported that when parasitoids are stored under optimum temperature, for example, between 0° and 7°C (e.g. Hofsvang & Hågvar 1977; Singh and Srivastava 1988; Colinet *et al.* 2006), insects may enter a quiescent state where their metabolic activities are decelerated in response to the adverse environmental conditions (Tauber *et al.* 1986; Lins 2013). However, when removed from storage they develop and behave normally (Leopold 1998). Second, eggs are more adaptable to the gradually decreasing temperature rather than cold shock treatments adopted by Abukhashim & Luff (1997). Many studies suppose that a pre-and post-acclimatization procedure can reduce injuries caused by prolonged exposure to low temperatures (Singh & Srivastava 1988; Leopold 1998; Levie *et al.* 2005; Colinet & Boivin 2011; Lins 2013). Third, compared to that tested in this study, eggs laid by the overwintering adults will survive better and can survive at least for four days or longer when temperature is  $\approx 5$  °C in spring, as eggs matured in mother's ovaries have experienced and thus adapted to the adverse environmental conditions.

Prolonged chilling required for terminating winter diapause is often reported (i.e., Tauber & Tauber 1976; Tauber *et al.* 1986; Xiao *et al.* 2013). To our best knowledge, chilling is more frequently reported to arrest rather than accelerate the development of immature stages, and most studies are relevant to mass production, storage, shipment and release of natural enemies in biological control programs (van Lenteren & Tommasini 2003; Lins 2013; Ismail *et al.* 2014). We found that egg chilling significantly 'accelerated' egg development with no significant difference found between chilling durations (Figure 2). These results may imply that eggs were able to develop at 5 °C for  $\leq 24$  hours but after which time the development was suspended.

In contrast to egg survival (i.e., hatching), chilling duration of eggs significantly affected the immature survival (Figure 1) and development (Table 1) indicating that immature stages are sensitive to cold stress; while prolonged exposure of eggs to chilling did not always had significant negative effects on immature survival (Figure 1) and development (Table 1). The mechanisms behind this phenomenon are mysterious. However, our results clearly show that chilling for  $\geq 48$  hours significantly decreased the survival of deutonymphs which may be the cause of significantly lower total immature survival rate detected for chilling durations of  $\geq 48$  hours (Figure 1).

Prolonged exposure to low temperatures associated with poor survival of immature stages is common (Hofsvang & Hågvar, 1977; Jones & Kunz 1998; Leopold 1998; Chen *et al.* 2008; Colinet & Boivin 2011; Mockett & Matsumoto 2014). According to Storey & Storey (1988), Rojas & Leopold (1996) and Leopold (1998), it is suggested that despite the 'normal' development of eggs, oxidative stress caused by build-up of reactive oxygen species in eggs might have occurred during the lengthy exposure to lower temperature, and such oxidative stress was passed into and caused injury to the subsequent immature stages. Many studies (Danks 1978; Bale 1987; Yocum *et al.* 1994;



Jones & Kunz 1998) demonstrated that temperatures below that for normal development may cause both immediate and delayed injury. Moreover, due to the longer cool period during early spring, *T. urticae* may suffer higher immature mortality in the fields than do under optimum environmental conditions in the laboratory. Therefore, our results expect that egg chilling for two days or longer during early spring will reduce the possibilities of population establishment and future outbreak in later seasons.

In spider mites, only the first mating results in fertilization (Potter & Wrensch 1978; Satoh *et al.* 2001; Oku 2008); and males usually develop faster than do females (Bounfour & Tanigoshi 2001; Riahi *et al.* 2013) in order to guard quiescent deutonymph females and mate at emergence (Potter *et al.* 1976; Satoh *et al.* 2001; Oku 2008, 2009). Our results show that egg chilling had no significant effect on female development, but chilling for 72 and 96 hours significantly delayed the emergence of male adults (Table 1). The delayed emergence in *T. urticae* males may result in the loss of opportunities to mate with emerged females before the latter disperse from the feeding sites. As *T. urticae* is arrhenotokous and haplodiploid species that only mated females can produce both male and female offspring (Macke *et al.* 2011), the accumulative number of unmated females in the cool spring may be a factor preventing the outbreaks of *T. urticae* in growing seasons.

The long-term influence of prolonged chilling of an immature stage on the female reproductive fitness is species specific, having significant negative effect (e.g., Jones & Kunz 1998; Mockett & Matsumoto 2014) or no significant effect (e.g., Chen *et al.* 2008, 2011). In the present study, the survival of immature stages (56.9% ~ 65.2%) in next generation was not significantly affected by egg chilling, and significant difference in longevity of male adults was only detected between chilling durations of 24 and 96 hours (Figure 3A). However, the significant difference in the reproductive performance of resultant *T. urticae* females (Table 2, Figure 3) indicates that the effect of chilling at the early stage (i.e., egg) of parental generation can extend to the resultant females. Although females started reproduction significantly earlier when parental eggs were chilled for  $\geq 48$  hours, this will not let to the early build-up of mite populations because reproductive output of those females was significantly lower (Table 2). The significantly lower reproductive output may be caused by the significantly shorter longevity of those females (Figure 3B) when parental eggs were chilled for longer duration. It is widely reported in insects that females will live shorter and lay fewer eggs if parental pupae are cold stored for a lengthy duration (e.g., Jones & Kunz 1998; Colinet *et al.*, 2006; Chen *et al.* 2008; Kidane *et al.* 2015).

In parasitoids prolonged storage of pupae at low temperatures resulting male-biased sex ratio has been reported for some species (e.g., Chen *et al.* 2008; Colinet & Hance 2010; Ismail *et al.* 2010; Lins 2013). The mechanisms resulting in the changes in sex ratio after storage have yet to be elucidated (Lins 2013). While Ismail *et al.* (2010) found in *Aphidius ervi* Haliday (Hymenoptera: Braconidae), males are more sensitive to cold storage than do females, suggesting that cold storage affects the quality of sperm before insemination. Our results show that the sex ratio of *T. urticae* is female-biased (Table 2) consistent with those reported by other researchers (Cone *et al.* 1986; Macke *et al.* 2011; Riahi *et al.* 2013). It may be concluded that prolonged chilling of parental eggs had no negative effect on male sperm quality. Inversely prolonged chilling of parental eggs increased female-biased sex ratio (Table 2), implying that prolonged chilling may improve sperm quality or males transfer more sperm during copulation, which is worth for further study. Alternately as *T. urticae* females can adjust sex allocation in response to egg size (larger eggs are more likely to be fertilized, Macke *et al.* 2011), thus it is assumed that females raised from eggs chilled for longer durations were better to use sperm to fertilize more relatively small eggs, as a compensative strategy due to their lower fecundity.

In conclusion, prolonged chilling of *T. urticae* eggs significantly decreased the survival of subsequent immature stages and suppressed the reproductive fitness of resultant females. Thus it is

supposed that prolonged low temperature during the early spring may be a factor limiting the probabilities of *T. urticae* establishing their population during the early season and the chance of outbreak during the growing season. Whereas *T. urticae* is an invasive species in China (Dong *et al.* 1987) and population dynamics of *T. urticae* usually show inter- and intra-population variations (Takafuji *et al.* 1991). Future studies on the seasonal phenology of inter- and intra-populations and the reproductive behavior of overwintering populations are helpful in understanding the field ecology and management of this pest.

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