

## **The Fitness of *Metaseiulus occidentalis* (Acari: Phytoseiidae) Adult Females after Different Intervals of Storage at Low Temperatures**

Authors: Yanar, Durdane, and Hoy, Marjorie A.

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# The fitness of *Metaseiulus occidentalis* (Acari: Phytoseiidae) adult females after different intervals of storage at low temperatures

Durdane Yanar<sup>1,\*</sup> and Marjorie A. Hoy<sup>2</sup>

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## Abstract

This study was carried out to determine the effects of temperature, relative humidity (RH), and an additional food source (honey) on the survival of *Metaseiulus occidentalis* (Nesbitt) (Acari: Phytoseiidae) females under cold-storage conditions. Survival was evaluated after 2, 4, and 8 wk at 7 or 10 °C under 75 or 95% RH. At the end of each cold-storage interval, surviving females were transferred to bean leaf discs with *Tetranychus urticae* Koch (Prostigmata: Tetranychidae) as prey, and their longevity, total number of eggs, and number of eggs each female produced per day were recorded. The highest survival rate (78.3%) was observed after 2 wk at 10 °C and 95% RH without honey in the tube. Survival was next highest (75%) after 2 wk at 7 °C and 95% RH without honey in tube. Survival was 76.7% at 7 °C with 95% RH without honey after 4 wk. Survival was low after 8 wk at both 7 or 10 °C, with 0 and 13.3% survival, respectively, at 95% RH. Adult longevity, the total number of eggs deposited, and the average number of eggs per female per day were not significantly different between females held for 2 or 4 wk at 7 or 10 °C at 95% RH with or without honey. Based on these results, *M. occidentalis* can be cold stored at 7 and 10 °C at 95% RH for up to 4 wk, but adding honey provided no benefit to the females.

Key Words: augmentative release; biological control; cold storage; fecundity; predator; survival rate

## Resumen

Se realizó este estudio para determinar los efectos de la temperatura, la humedad relativa (HR) y una fuente de alimentación adicional (miel) sobre la sobrevivencia de hembras de *Metaseiulus occidentalis* (Nesbitt) (Acari: Phytoseiidae) en condiciones de frío. Se evaluó la sobrevivencia después de 2, 4 y 8 semanas a los 7 o 10 °C bajo 75 o 95% de HR. Al final de cada intervalo de almacenamiento en frío, hembras sobrevivientes fueron trasladadas a discos de hojas de frijol con *Tetranychus urticae* Koch (Prostigmata: Tetranychidae) como presa y se registró su longevidad, el número total de huevos, y el número de huevos por hembra producida por día. Se observó la tasa de sobrevivencia más alta (78.3%) después de 2 semanas a 10 °C y 95% HR sin miel en el tubo. La siguiente sobrevivencia fue más alta (75%) fue después de 2 semanas a 7 °C y 95% HR sin miel en el tubo. La sobrevivencia fue 76.7% a 7 °C con 95% HR sin miel después de 4 semanas. La sobrevivencia fue baja después de 8 semanas en tanto 7 o 10 °C a 95% de HR, con 0 y 13.3% de sobrevivencia, respectivamente. La longevidad de los adultos, el número total de huevos depositados, y el número promedio de huevos por hembra por día no fueron significativamente diferentes entre hembras mantenidos por 2 o 4 semanas a 7 o 10 °C a 95% de humedad relativa con o sin miel. Basándose en estos resultados, *M. occidentalis* pueden ser almacenados en frío a 7 y 10 °C a 95% de humedad relativa hasta 4 semanas, pero la adición de miel no proporciona ningún beneficio para las hembras.

Palabras Clave: liberación aumentativa; control biológico; almacenamiento en frío; fecundidad; depredadores; tasa de sobrevivencia

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*Metaseiulus* (= *Galendromus* or *Typhlodromus*) *occidentalis* (Nesbitt) (Acari: Phytoseiidae) is an important and effective predator of pest mites in agricultural crops around the world (Hoy 2011). *Metaseiulus occidentalis* reproduces very quickly, and there may be 8–10 generations per year in the field (Beers et al. 1993). This predator is sometimes used in augmentative releases and is available commercially, including some pesticide-resistant strains (Hoy et al. 1982; Bruce-Oliver & Hoy 1990; Hunter 1997; Luczynski et al. 1997, 2008; Hoy 2011).

Storage of mass-reared phytoseiids is an important issue for commercial suppliers. Commercial producers often are required to produce large numbers of phytoseiids during specific times of the year. This can be difficult, so commercial suppliers are interested in the possibility of

stockpiling predatory mites for shipment during peak times. Storage for more than one month is considered long-term storage (Leopold 1998; van Lenteren & Tommasini 1999). *Amblyseius cucumeris* (Oudemans) larvae or adults can be stored at 8–9 °C for 10 wk and *Phytoseiulus persimilis* Athias-Henriot larvae or adults can be stored at 7.5 °C for 4 wk (Leopold 1998), although chilling causes high rates of mortality during storage (Luczynski et al. 2008). The availability of food also affects the survival of predatory mites (Morewood 1992; Bogdanov et al. 2008; Gotoh & Tsuchiya 2009). The ability to store *M. occidentalis* could increase its use in augmentative biological control.

The objectives of this study were to investigate the storage potential of *M. occidentalis*. Data on mortality of *M. occidentalis* females

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<sup>1</sup>Plant Protection Department, Faculty of Agriculture, Gaziosmanpasa University, 60240 Tokat, Turkey

<sup>2</sup>Department of Entomology and Nematology, University of Florida, Gainesville, FL 32611, USA

\*Corresponding author; E-mail: durdane.yanar@gop.edu.tr

held at 7 or 10 °C at 75 or 95% RH were recorded. In addition, the survival rate for females provided with honey or no honey during storage was evaluated. Longevity and the reproductive capacity of females after removal from cold storage were evaluated, as were the hatch rates and developmental success of their larvae. All comparisons were made with females held at standard laboratory conditions.

Materials and Methods

COLONY SOURCE AND CULTURE METHODS

A freshly collected colony of *M. occidentalis* was obtained from an organic apple orchard in Washington State (USA) in Jun 2012 and all experiments were conducted between Jul and Nov 2012 at the Department of Entomology and Nematology, University of Florida, Florida, USA. Cultures of *M. occidentalis* were maintained on 7.5 × 7.5 cm paraffin-coated black paper placed on water-soaked cotton in 13 × 13 cm plastic trays, and fed all stages of *T. urticae* under laboratory conditions of 25 °C and 45 ± 5% RH and a 16:8 h L:D photoperiod.

EXPERIMENTAL DESIGN

Newly emerged females of *M. occidentalis* were allowed to mate and feed on all stages of *T. urticae* for 24 h, then were placed into 4 × 1 cm glass tubes for the 8 treatments for 2, 4, and 8 wk cold storage. The treatments were 7 °C with honey, 75 ± 5% RH; 7 °C without honey, 75 ± 5% RH; 7 °C with honey, 95 ± 5% RH; 7 °C without honey, 95 ± 5% RH; 10 °C with honey, 75 ± 5% RH; 10 °C without honey, 75 ± 5% RH; 10 °C with honey, 95 ± 5% RH; 10 °C without honey, 95 ± 5% RH. Honey was placed on a 2 mm diameter filter paper disc and blotted to reduce the likelihood that females would become trapped in the honey. The tubes were held for 2, 4, or 8 wk at 7 or 10 °C under 75 ± 5% or 95 ± 5% RH under a 16:8 h L:D photoperiod in growth chambers. Five females were placed into each vial, which was sealed with parafilm (American Can Co., Greenwich, Connecticut, USA) that had 5 tiny holes produced by minuten pins to ensure airflow into

and out of the tubes. The vials were placed into sealed chambers in which saturated salt solution (sodium chloride) was present in plastic trays in the bottom of the container to maintain the RH at 75 ± 5% (Winston & Bates 1960). Saturated water in trays at the bottom of the sealed container maintained the 95 ± 5% RH.

After each cold-storage interval, each surviving female was isolated on a 19 mm diameter pinto bean (*Phaseolus vulgaris* L.; Fabales: Fabaceae) leaf disc placed on water-soaked cotton in plastic trays (13 × 13 cm) under laboratory conditions of 25 °C, 45 ± 5% RH, and a 16:8 h L:D photoperiod with all stages of *T. urticae* prey.

The total percentage of females surviving was recorded after 2, 4, or 8 wk. Adults were observed daily until they died after removal from cold storage, and the numbers of eggs deposited per surviving female per day were recorded daily. Females that were not held in cold storage and were reared on all stages of live *T. urticae* under laboratory conditions of 25 °C, 45 ± 5% RH, and a 16:8 h L:D photoperiod were used as controls to estimate any fitness costs due to cold storage. There were 5 females per glass tube per treatment (8 treatments) and 12 replicates of each treatment.

STATISTICAL ANALYSES

The data were analyzed by the SPSS 17 statistical program (SPSS Inc. 2008) using a general linear model to compare the effects of temperature, RH, honey in the tube, and weeks in cold storage. ANOVA was used to compare means and Tukey’s test was used to separate means. Chi-square was used to evaluate the effect of RH and cold-storage time, temperature, and honey on mortality.

Results

EFFECT OF STORAGE TIME ON SURVIVAL OF *M. OCCIDENTALIS* FEMALES

Survival of adult females at the 3 different storage periods (2, 4, and 8 wk) was significantly different ( $\chi^2 = 369.9$ ; df = 2; 0.001 < *P* <

**Table 1a.** Percentage survival of *Metaseiulus occidentalis* females fed honey or no honey after 2 wk of storage at 7 or 10 °C, and their longevity and fecundity compared with females that had not been cold stored.

| 25 °C (Control)                 |                |                |                |                |
|---------------------------------|----------------|----------------|----------------|----------------|
| % female survival               | 100            |                |                |                |
| Longevity (mean ± SE) (d)       | 10.9 ± 0.4 a   |                |                |                |
| Total eggs/female (mean ± SE)   | 15.0 ± 0.9 ab  |                |                |                |
| No. eggs/female/day (mean ± SE) | 1.3 ± 0.1 a    |                |                |                |
| Two weeks of storage at         | 75% RH         |                | 95% RH         |                |
|                                 | Honey          | No honey       | Honey          | No honey       |
| 7 °C                            |                |                |                |                |
| % female survival               | 45.0 ± 6.1 cd  | 48.3 ± 5.2 bcd | 70.0 ± 5.2 abc | 75.0 ± 6.1 ab  |
| Longevity (mean ± SE) (d)       | 12.2 ± 1.0 a   | 12.4 ± 1.0 a   | 11.7 ± 0.7 a   | 10.5 ± 0.8 ab  |
| Total eggs/female (mean ± SE)   | 13.9 ± 2.2 abc | 12.7 ± 1.9 abc | 19.9 ± 1.7 a   | 12.4 ± 1.5 abc |
| No. eggs/female/day (mean ± SE) | 1.1 ± 0.1 a    | 1.0 ± 0.1 ab   | 1.6 ± 0.1 a    | 1.1 ± 0.1 ab   |
| 10 °C                           |                |                |                |                |
| % female survival               | 68.3 ± 8.3 abc | 40.0 ± 7.4 ed  | 70.0 ± 6.3 abc | 78.3 ± 5.2 a   |
| Longevity (mean ± SE) (d)       | 12.2 ± 0.7 a   | 11.7 ± 1.0 a   | 11.4 ± 0.8 a   | 10.3 ± 0.6 ab  |
| Total eggs/female (mean ± SE)   | 14.3 ± 1.6 abc | 17.5 ± 2.5 a   | 12.8 ± 1.7 abc | 11.8 ± 1.6 abc |
| No. eggs/female/day (mean ± SE) | 1.1 ± 0.1 a    | 1.4 ± 0.1 a    | 1.1 ± 0.1 a    | 1.1 ± 0.1 a    |

Means within the same row for % female survival, longevity after removal from cold, total eggs per female, and number of eggs/female/day followed by the same letter are not significantly different (*P* > 0.05, Tukey test).

**Table 1b.** Percentage survival of *Metaseiulus occidentalis* females fed honey or no honey after 4 wk of storage at 7 or 10 °C, and their longevity and fecundity.

| Four weeks of cold storage      | 75% RH          |                | 95% RH          |                 |
|---------------------------------|-----------------|----------------|-----------------|-----------------|
|                                 | Honey           | No honey       | Honey           | No honey        |
| <b>7 °C</b>                     |                 |                |                 |                 |
| % female survival               | 14.6 ± 6.1 ef   | 12.7 ± 4.1 f   | 61.7 ± 5.2 abcd | 76.7 ± 6.0 a    |
| Longevity (mean ± SE) (d)       | 8.5 ± 4.5 ab    | 12.5 ± 0.3 a   | 10.6 ± 0.5 ab   | 10.8 ± 0.5 ab   |
| Total eggs/female (mean ± SE)   | 12.0 ± 11.0 abc | 10.8 ± 4.0 abc | 13.7 ± 1.1 abc  | 12.8 ± 1.3 abc  |
| No. eggs/female/day (mean ± SE) | 1.0 ± 0.8 ab    | 0.8 ± 0.3 abc  | 1.3 ± 0.1 a     | 1.2 ± 0.1 a     |
| <b>10 °C</b>                    |                 |                |                 |                 |
| % female survival               | 14.6 ± 6.7 ef   | 9.1 ± 4.9 f    | 66.7 ± 3.8 abcd | 65.0 ± 7.0 abcd |
| Longevity (mean ± SE) (d)       | 10.6 ± 0.8 ab   | 10.8 ± 1.0 ab  | 10.9 ± 0.3 ab   | 11.0 ± 0.4 a    |
| Total eggs/female (mean ± SE)   | 10.6 ± 1.7 abc  | 14.2 ± 2.6 abc | 11.1 ± 1.4 abc  | 13.5 ± 1.3 abc  |
| No. eggs/female/day (mean ± SE) | 1.0 ± 0.2 ab    | 1.3 ± 0.2 a    | 1.0 ± 0.1 ab    | 1.2 ± 0.1 a     |

Means within same row for % female survival, longevity after removal from cold, total eggs per female, and number of eggs/female/day followed by the same letter are not significantly different ( $P > 0.05$ , Tukey test).

0.01) (Table 1a,b,c). The survival rates decreased as the storage time increased irrespective of whether food (honey) was provided or not. For instance, after 2 wk at 7 °C with 75% RH, the survival rate was 45.0 or 48.3%, respectively, with or without honey in the tubes (Table 1a). After 4 wk at 7 °C and 75% RH, survival was reduced to 14.6 or 12.7%, respectively, with or without honey (Table 1b). After 8 wk at 7 °C and 75% RH, none of the females survived with or without honey (Table 1c). Survival between weeks was significantly different for 2, 4, and 8 wk at 7 °C and 75% RH ( $F = 24.64$ ;  $df = 5, 64$ ;  $P < 0.05$ ) (Table 1a,b,c).

After 2 wk at 7 °C and 95% RH, survival was 70.0 or 75.0%, respectively, with or without honey (Table 1a). After 4 wk, survival was 61.7 or 76.7%, respectively, with or without honey (Table 1b). After 8 wk, survival was reduced to 11.7 or 13.3%, respectively, with or without honey (Table 1c). The survival of females held for 2 and 4 wk was not significantly different at 7 °C and 95% RH, but survival after 8 wk at 7 °C and 95% RH was significantly different from that of 2 and 4 wk ( $F = 29.19$ ;  $df = 5, 66$ ;  $P < 0.05$ ) (Table 1a,b,c).

After 2 wk of cold storage at 10 °C and 75% RH, the survival rate was 68.3 or 40.0%, respectively, with or without honey (Table 1a). After 4 wk at 10 °C and 75% RH with or without honey, survival was 14.6 or 9.1%, respectively (Table 1b). After 8 wk, no females survived with or without honey. Survival between weeks was significantly different for 2, 4, and 8 wk at 10 °C and 75% RH ( $F = 23.38$ ;  $df = 5, 64$ ;  $P < 0.05$ ) (Table 1a,b,c).

After 2 wk at 10 °C and 95% RH, the survival rate was 70.0 or 78.3%, respectively, with or without honey (Table 1a). After 4 wk, with or without honey, survival was 66.7 or 65.0%, respectively (Table 1b). After 8 wk, survival was 0 or 1.7%, respectively, with or without honey (Table 1c). The survival of females held for 2 and 4 wk was not significantly different at 10 °C and 95% RH, but the survival rate after 8 wk at 10 °C and 95% RH was significantly different from those after 2 and 4 wk ( $F = 58.70$ ;  $df = 5, 66$ ;  $P < 0.05$ ).

#### EFFECT OF RH ON SURVIVAL OF *M. OCCIDENTALIS* FEMALES

Two different RH conditions (75 and 95%) were evaluated, and the survival rates were significantly different ( $\chi^2 = 120.6$ ;  $df = 1$ ;  $0.001 < P < 0.01$ ). The 95% RH significantly increased the survival of *M. occidentalis* (61.7–76.7% survival) when compared with 75% RH (12.7–48.3%) in the 2 and 4 wk storage treatments at 7 °C ( $F = 179.7$ ;  $df = 1$ ;  $P < 0.05$ ) (Table 1a,b). The 95% RH allowed a few survivors after 8 wk (11.7–13.3%) compared with no survival under the 75% RH conditions (Table 1c).

The 95% RH also significantly increased the survival of *M. occidentalis* females when compared with 75% RH in the 2 and 4 wk treatments at 10 °C ( $F = 179.7$ ;  $df = 1$ ,  $P < 0.05$ ) (Table 1a,b). The 95% RH allowed a few survivors after 8 wk at 10 °C compared with no survival at 75% RH at 10 °C (Table 1c). Thus, in addition to storage time, RH is important in the survival rate of *M. occidentalis* females.

**Table 1c.** Percentage survival of *Metaseiulus occidentalis* females fed honey or no honey after 8 wk of storage at 7 or 10 °C, and their longevity and fecundity.

| Eight weeks cold storage        | 75% RH      |             | 95% RH        |               |
|---------------------------------|-------------|-------------|---------------|---------------|
|                                 | Honey       | No honey    | Honey         | No honey      |
| <b>7 °C</b>                     |             |             |               |               |
| % female survival               | 0.0 ± 0.0 f | 0.0 ± 0.0 f | 11.7 ± 4.6 f  | 13.3 ± 6.7 ef |
| Longevity (mean ± SE) (d)       | —           | —           | 4.4 ± 0.3 b   | 9.3 ± 1.4 ab  |
| Total eggs/female (mean ± SE)   | —           | —           | 0.4 ± 0.3 c   | 9.7 ± 3.4 abc |
| No. eggs/female/day (mean ± SE) | —           | —           | 0.1 ± 0.1 abc | 0.9 ± 0.3 abc |
| <b>10 °C</b>                    |             |             |               |               |
| % female survival               | 0.0 ± 0.0 f | 0.0 ± 0.0 f | 0.0 ± 0.0 f   | 1.7 ± 1.7 f   |
| Longevity (mean ± SE) (d)       | —           | —           | —             | 6.5 ± 2.5 ab  |
| Total eggs/female (mean ± SE)   | —           | —           | —             | 2.0 ± 2.0 bc  |
| No. eggs/female/day (mean ± SE) | —           | —           | —             | 0.2 ± 0.2 abc |

Means within same row for % female survival, longevity after removal from cold, total eggs per female, and number of eggs/female/day followed by the same letter are not significantly different ( $P < 0.05$ , Tukey test).

EFFECT OF TEMPERATURE ON SURVIVAL OF *M. OCCIDENTALIS* FEMALES

Two temperature conditions (7 and 10 °C) were tested, but survival was not significantly different for the different cold-storage intervals ( $\chi^2 = 0.25$ ;  $df = 1$ ;  $0.1 > P > 0.05$ ). At 7 °C, the survival of *M. occidentalis* females held at 75% RH for 2 wk was not different with or without honey ( $F = 0.17$ ;  $df = 1$ ;  $P > 0.05$ ) (Table 1a). However, survival of females held at 10 °C at 75% RH for 2 wk was significantly different if provided honey ( $F = 6.48$ ;  $df = 1$ ;  $P < 0.05$ ) (Table 1a). Survival of females held at 95% RH at 7 or 10 °C with or without honey did not differ significantly ( $F = 0.51$ ;  $df = 3$ ;  $P > 0.05$ ).

The survival rates of females held at 7 and 10 °C and 95% RH were different statistically after 4 wk ( $F = 28.19$ ;  $df = 7$ ;  $P < 0.05$ ). The survival rates of females held at 7 and 10 °C at 75% RH were not different statistically ( $F = 0.22$ ;  $df = 3$ ;  $P > 0.05$ ) (Table 1b). After 8 wk there was some survival at 7 and 10 °C at 95% RH and the temperature did not have a significant effect on survival ( $F = 3.75$ ;  $df = 7$ ;  $P > 0.05$ ) (Table 1c).

EFFECT OF HONEY ON SURVIVAL OF *M. OCCIDENTALIS* FEMALES

Females of *M. occidentalis* were provided *T. urticae* prey during their preovipositional and mating intervals, then were placed into glass tubes with honey-impregnated filter paper or no honey. Results show no significant difference in survival ( $\chi^2 = 0.003$ ;  $df = 1$ ;  $0.1 > P > 0.05$ ) (Table 1a,b,c). These results show that the survival of *M. occidentalis* females was dependent more on RH and cold storage time than on temperature (7 or 10 °C) and food availability (honey or no honey) during cold storage.

LONGEVITY AND FECUNDITY OF *M. OCCIDENTALIS* FEMALES AFTER COLD STORAGE

Females that survived cold storage were evaluated for their longevity and egg-laying capacity (Table 1a,b,c). We also evaluated the ability of eggs to hatch and the rate of successful development of the larvae to adulthood. Significant differences were observed between the longevities of the control and cold-stored *M. occidentalis* females ( $F = 1.8$ ;

$df = 19$ ;  $P < 0.05$ ) and their egg-laying capacities (total number of eggs/female) ( $F = 2.8$ ;  $df = 19$ ;  $P < 0.05$ ) (Table 1a,b,c).

However, females held for 2 or 4 wk at 75 or 95% RH and 7 or 10 °C with or without honey and 8 wk at 95% RH at 7 °C without honey were not significantly different in their longevities compared with control females (8.5–12.5 d) (Table 1a,b,c). Only survival of females held at 95% RH at 7 °C without honey was significantly different from control females (4.4 d).

Females held for 2 or 4 wk at 75 or 95% RH at 7 or 10 °C with or without honey and 8 wk at 95% RH and 7 °C without honey were not significantly different in the mean number of eggs deposited (9.7–19.9 eggs/female) (Table 1a,b,c). Females held for 8 wk at 95% RH and 7 or 10 °C without honey produced significantly fewer eggs (0.4–2.0 eggs/female) than control females. Females held at 75% RH at 7 or 10 °C with or without honey did not survive; so we could not examine their longevity or fecundity.

HATCH OF EGGS LAID BY COLD-STORED *M. OCCIDENTALIS* FEMALES

The eggs laid by females stored for 2 or 4 wk did not show statistically significant differences in hatch rates. However, eggs produced by females held for 8 wk in cold storage had a significantly reduced hatch rate compared with eggs produced by females held for 2 or 4 wk ( $F = 3.2$ ;  $df = 19$ ;  $P < 0.05$ ) (Table 2a,b,c). Temperature ( $F = 2.4$ ;  $df = 1$ ;  $P > 0.05$ ), presence of honey ( $F = 0.6$ ;  $df = 1$ ;  $P > 0.05$ ), and RH ( $F = 0.9$ ;  $df = 1$ ;  $P > 0.05$ ) did not affect hatch rates of eggs laid by *M. occidentalis* females held for 2 and 4 wk in the cold (Table 2a,b). The eggs laid by females held for 2 or 4 wk of cold storage at 75 or 95% RH and 7 or 10 °C with and without honey and 8 wk at 95% RH and 7 °C without honey were not significantly different in egg hatchability compared with those laid by control females (81.2–99.0% egg hatch) ( $F = 3.2$ ;  $df = 19$ ;  $P < 0.05$ ) (Table 2a,b,c).

SURVIVAL OF LARVAE HATCHING FROM EGGS DEPOSITED BY COLD-STORED FEMALES

The developmental success of the larvae that hatched from eggs deposited by cold-stored females was not significantly different from

**Table 2a.** Egg hatch rates and survival of larvae to adults of eggs laid by *Metaseiulus occidentalis* females that had survived 2 wk of cold storage compared with females that had not been cold stored.

| 25 °C Control                              |                 |                |               |                |
|--|-----------------|----------------|---------------|----------------|
| % egg hatch (mean ± SE)                    | 81.8 ± 13.4 abc |                |               |                |
| % survival of larvae to adult (mean ± SE)  | 85.3 ± 2.2 ab   |                |               |                |
| Sex ratio (female: male)                   | 1.2:1           |                |               |                |
| Two weeks cold storage                     | 75% RH          |                | 95% RH        |                |
|  | Honey           | No honey       | Honey         | No honey       |
| <b>7 °C</b>                                |                 |                |               |                |
| % egg hatch (mean ± SE)                    | 82.5 ± 3.8 abc  | 88.6 ± 2.4 ab  | 86.2 ± 3.4 ab | 81.2 ± 2.6 abc |
| % survival of larvae to adults (mean ± SE) | 94.4 ± 6.3 ab   | 85.7 ± 4.4 ab  | 88.1 ± 4.6 ab | 90.8 ± 2.4 ab  |
| Sex ratio (female: male)                   | 1.1:1           | 1.1:1          | 1:1           | 1.3:1          |
| <b>10 °C</b>                               |                 |                |               |                |
| % egg hatch (mean ± SE)                    | 81.3 ± 2.9 abc  | 81.3 ± 2.6 abc | 86.2 ± 3.4 ab | 89.3 ± 2.6 ab  |
| % survival of larvae to adults (mean ± SE) | 91.3 ± 2.9 ab   | 90.8 ± 2.4 ab  | 87.3 ± 4.5 ab | 88.28 ± 3.2 ab |
| Sex ratio (female: male)                   | 1.16:1          | 1.26:1         | 1.02:1        | 0.9:1          |

Means within a row for % egg hatch, survival of larvae to adults, and sex ratio followed by the same letter are not significantly different ( $P > 0.05$ , Tukey test).



**Table 2b.** Egg hatch rates and survival of larvae to adults of eggs laid by *Metaseiulus occidentalis* females that had survived 4 wk of cold storage.

| Four weeks cold storage                    | 75% RH          |                | 95% RH         |                |
|--|-----------------|----------------|----------------|----------------|
|  | Honey           | No honey       | Honey          | No honey       |
| <b>7 °C</b>                                |                 |                |                |                |
| % egg hatch (mean ± SE)                    | 84.8 ± 9.3 ab   | 80.7 ± 6.6 abc | 76.0 ± 4.0 bc  | 83.4 ± 3.9 ab  |
| % survival of larvae to adults (mean ± SE) | 90.7 ± 6.2 ab   | 96.3 ± 3.7 a   | 78.0 ± 4.1 ab  | 81.0 ± 5.0 ab  |
| Sex ratio (female: male)                   | 1.1:1           | 1:1            | 1:1            | 0.8:1          |
| <b>10 °C</b>                               |                 |                |                |                |
| % egg hatch (mean ± SE)                    | 82.2 ± 13.4 abc | 99.0 ± 7.1 a   | 81.0 ± 2.6 abc | 81.3 ± 2.5 abc |
| % survival of larvae to adults (mean ± SE) | 78.9 ± 10.2 ab  | 86.7 ± 6.9 ab  | 79.8 ± 3.2 ab  | 73.3 ± 3.5 ab  |
| Sex ratio (female: male)                   | 1.4:1           | 1.9:1          | 0.5:1          | 0.8:1          |

Means within a row for % egg hatch, survival of larvae to adults, and sex ratio followed by the same letter are not significantly different ( $P > 0.05$ , Tukey test).

the survival rates of progeny produced by control females held at 25 °C ( $F = 2.3$ ;  $df = 19$ ;  $P > 0.01$ ) (Table 2a,b,c). The lowest survival rate was 67.6% when females had been held at 95% RH at 7 or 10 °C without honey. The highest survival rate was 96.3%, in larvae from females held at 75% RH at 7 °C without honey.

## Discussion

Cold-storage time had an important effect on the survival of *M. occidentalis* females. In this study, 2 and 4 wk of cold storage at 7 or 10 °C did not appear to have a significant effect on survival or fitness for *M. occidentalis* females or their progeny, but storage for 8 wk was detrimental. The other factor affecting survival during cold storage was RH. At 75% RH, survival was 14.6% in the presence of honey and 12.7% without honey at the end of 4 wk at 7 °C. By contrast, at 95% RH, the survival rate of females provided either with or without honey increased to 61.7 or 76.7%, respectively, at the end of 4 wk at 7 °C.

Previous studies indicate that low RH is not suitable for cold storage of other species of phytoseiids (Ghazy et al. 2012). The survival of *P. persimilis* was 97% after 4 wk at 7.5 °C and 80% after 6 wk at 7.5 °C when mites had been provided with *T. urticae* eggs (Morewood 1992). The survival rate of *M. occidentalis* (61.7–76.7% at 95% RH after 4 wk) was lower than the survival rate of *P. persimilis* (Morewood 1992), likely due to species differences. Hamamura et al. (1978) found that survival of *P. persimilis* held at 5, 7.5, or 10 °C at 95% RH after 12 d was 72, 76, or 84%, respectively. But after 25 d, the survival rates were 20, 28, or 12%, respectively at 5, 7.5, or 10 °C. Their survival rates for *P. persimilis* after 12 d were similar to our results for *M. occidentalis* (70.0–78.3% survival at 95% RH). However, their survival rates for *P. persimilis* after 25 d were lower than our results for *M. occidentalis* after 4 wk (62.7–76.7% survival at 95% RH). Hamamura et al.

(1978) found that 100% RH was more suitable than 95% RH for survival of *P. persimilis* at 7.5 and 10 °C (92 and 96% at the end of 25 d).

Adult longevities of *M. occidentalis* were not significantly different after 2 and 4 wk whether the mites were provided honey or not. However, Hamamura et al. (1978) found that honey-fed *P. persimilis* females had greater longevities than *T. urticae*-fed females after cold storage for 30 and 50 d.

Morewood (1992) found that *P. persimilis* females, within a few days after their return to room temperature, laid eggs at the same rate as females taken directly from rearing cultures. In this study, surviving *M. occidentalis* females held for 2 or 4 wk also laid eggs at the same rate as control females. Nicoli & Galazzi (1998) found that survival of predatory mites during storage decreased, but the surviving females showed greater longevity, fertility, and fecundity after isolation at 25 °C. Our results were similar to those of Nicoli & Galazzi (1998): Surviving *M. occidentalis* females had good longevity and comparable fecundity rates if held in cold storage for 2 or 4 wk.

*Metaseiulus occidentalis* females, taken from the general colony and fed *T. urticae* eggs and mobile stages, laid an average of 15.0 eggs per female, whereas the cold-stored females laid 13.7 or 12.8 eggs when fed either honey or no honey, respectively, at 95% RH after 4 wk. Egg-laying capacity observed with control females and cold-stored females in our study were somewhat different from those obtained by Akyazi & Hoy (2013) using a different *M. occidentalis* colony. Their colony females laid a mean total of 22.8 eggs per female when fed *T. urticae* eggs, whereas females laid 10.7 eggs per female when fed only *T. urticae* active stages. In this study, we fed females both active stages and eggs, so differences in egg deposition may be due to the prey type provided or to the strain of *M. occidentalis* evaluated.

The data obtained on fitness of cold-stored *M. occidentalis* will be useful for commercial producers and for pest-management specialists

**Table 2c.** Egg hatch rates and survival of larvae to adults of eggs laid by *Metaseiulus occidentalis* females that had survived 8 wk of cold storage.

| Eight weeks cold storage                   | 75% RH |          | 95% RH        |               |
|--|--------|----------|---------------|---------------|
|  | Honey  | No honey | Honey         | No honey      |
| <b>7 °C</b>                                |        |          |               |               |
| % egg hatch (mean ± SE)                    | —      | —        | 83.5 ± 3.9 ab | 61.7 ± 4.7 c  |
| % survival of larvae to adults (mean ± SE) | —      | —        | 67.6 ± 5.1 b  | 87.9 ± 4.5 ab |
| Sex ratio (female: male)                   | —      | —        | 0.9:1         | 0.7:1         |
| <b>10 °C</b>                               |        |          |               |               |
| % egg hatch (mean ± SE)                    | —      | —        | —             | 71.4 ± 9.6 bc |
| % survival of larvae to adult (mean ± SE)  | —      | —        | —             | 88.1 ± 5.7 ab |
| Sex ratio (female: male)                   | —      | —        | —             | 1:1           |

Means within a row for % egg hatch, survival of larvae to adults, and sex ratio followed by the same letter are not significantly different ( $P > 0.05$ , Tukey test).

wishing to use these predators in augmentative releases. In addition, these data will help scientists maintain colonies in research laboratories when there are crises with prey production. Because *M. occidentalis* is an obligatory predator, valuable colonies can crash during periods when prey becomes scarce. Storing this species at 7 or 10 °C at 95% RH up to 4 wk until prey colonies are available will allow valuable colonies to be maintained.

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