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Performance of *Orius insidiosus* after storage, exposure to dispersal material, handling and shipment processes

Vanda Helena Paes BUENO[1], Lívia Mendes CARVALHO[2], Joop C. VAN LENTEREN[3]
[1]*Departamento de Entomologia, Universidade Federal de Lavras, MG, Brasil*
[2]*Empresa de Pesquisa Agropecuária Minas Gerais, São João Del Rei, MG, Brazil*
[3]*Laboratory of Entomology, Wageningen University, The Netherlands*

Abstract

Storage, handling and shipment procedures are important factors influencing the quality of biological control agents. This study aimed to evaluate biological parameters and performance of *Orius insidiosus* (Say) after different storage periods at low temperatures, after exposure to different dispersal materials in containers, and after handling the predator during the shipment and delivery processes. Storage periods were 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 days at temperatures of 5, 8, 10 and 12 ± 1 °C, RH 70 ± 10% and under continuous scotophase. A mix of 75% adults and 25% 5th instar nymphs of *O. insidiosus* was kept in plastic containers (200 mL) for a 72 h period, supplied with eggs of *Anagasta kuehniella* (Zeller) as food, farmer's friend inflorescence (*Bidens pilosa* L.) as oviposition substrate and source of moisture, and one of the following dispersal materials: vermiculite + rice hulls (1:1), vermiculite, folded paper towels, sawdust, and coffee husk. Also, similar mixes of nymphs and adults were exposed to a 72 h shipment and delivery process. We found that *O. insidiosus* can be stored up to 10 days at 8 °C without loss of quality. Interestingly, storage of mated female predators results in a much higher fecundity post-storage than storage of virgin females. Vermiculite + rice hulls was by far the best dispersal material, and shipment of the predators by post during 72 h in Styrofoam boxes with plastic containers with vermiculite + rice hulls and *A. kuehniella* eggs did not negatively affect their survival and predation capacity. Our results can be used in planning mass-rearing and shipment, and to improve the quality of the predator *O. insidiosus* by using the right storage temperature, storage period and dispersal material.

Key words: augmentative biological control, mass rearing, storage temperature, type of dispersal material, predator, virgin females.

Introduction

The commercialization of biological control agents, including predatory Heteroptera, has strongly increased worldwide during the last decades (Bolckmans and Belda, 2008; Bale *et al*., 2008; van Lenteren, 2012). The heteropteran predator *Orius insidiosus* (Say) is mass-reared and used in commercial biological control in North America since 1985 (van Lenteren, 2012). Studies have demonstrated the effectivity of *O. insidiosus* against the western flower thrips *Frankliniella occidentalis* (Pergande) in greenhouse crops such as chrysanthemum (Silveira *et al*., 2004) and gerbera (Carvalho *et al*., 2008) in Brazil.

Internal and external consumer markets influence the reduction of pesticide in production of food and flowers in Latin America, and, as a result, the commercialization of biological control agents is increasing (van Lenteren and Bueno, 2003; van Lenteren, 2012). An example is the mass-rearing of *O. insidiosus*, a potential and viable activity in Brazil (Mendes *et al*., 2005a; Bueno, 2009), but also a promising candidate for biological control in other Latin American countries (Bueno and van Lenteren, 2012).

The success of commercial augmentative biological control depends, among other things, on the storage period, the type of storage and dispersal material, on the shipment method and the method of release of the natural enemy within the crop. Arthropod natural enemies need protection against extreme temperature and humidity, and also against mechanic injury when under transportation. Transportation of natural enemies to the fields or greenhouse release areas far away from the production facilities often creates problems for the biological control industry (van Lenteren and Tommasini, 2003). Particularly in Brazil, with often very large distances between the facilities where mass-rearing occurs and the crops where they are released, appropriate shipping conditions and care during transportation is essential.

Further, for biological companies, is important to be able to store natural enemies at low temperature without causing drastic negative effects on their quality (Denlinger and Lee, 1998; Leopold, 1998; Etzel and Legner, 1999; Bolckmans and Belda, 2008; Colinet and Boivin, 2011). Possibilities for storing mass-reared beneficial insects are important because the demand for these agents is often periodic and unpredictable. An appropriate temperature for cold storage of *Orius laevigatus* (Fieber) appeared to be 10 °C; this predator could be stored for 36 days without quality loss and with adult survival of 70% (JeongHwan *et al*., 2009). Eggs of *Orius sauteri* (Poppius) can be stored for up to 7 days at temperatures of 7.5 °C and 12.5 °C without adverse effects (Murai *et al*., 2001), and adults *O. insidiosus* maintain an acceptable longevity and reproduction rate after storage in diapause for up to 8 weeks at 15 °C (10:14 L:D) (Ruberson *et al*., 1998). Knowledge and use of the diapause syndrome of predators (Ruberson *et al*., 1998) and parasitoids (van Lenteren and Tommasini, 2003) can provide a natural mechanism for their effective long-term. However, most of this work has not yet led to practical applications, although with some positive exceptions like for *O. insidiosus* (van Lenteren and Tommasini, 2003).

Current methods for mass production of *Orius* predators are still relatively expensive (Bonte and De Clercq, 2010) and the development of an effective storage method is crucial to decrease rearing costs in order to meet the requirements for good planning for a mass-production unit and because of the difficulty of accurately prediction demand from clients (both delivery dates and quantities) (van Lenteren and Tommasini, 2003). In addition to possibilities for storage, the development of a proper storage or dispersal material is also important. The logistics of shipment and delivery of natural enemies to the field or greenhouse release sites remains another major problem for their commercialization (van Lenteren and Tommasini, 2003; Luczynski *et al.*, 2007).

Orius species are usually sold as adults or as a mix of 5th nymphs and adults, and the quantities inside the container vary with the producer of this natural enemy. *Orius* species, including *O. insidiosus*, can exhibit cannibalism when kept at high densities (van den Meiracker, 1999), when exposed to low quantities of food (Tommasini *et al*., 2002), but also when food is available in the container used for storage and transport (van Lenteren and Tommasini, 2003). In order to reduce the risk of cannibalism, hiding-places are usually provided in the dispersal containers in the form of paper, buckwheat and vermiculite (van Lenteren and Tommasini, 2003; Bolckmans and Belda, 2008). If the conditions within the containers are unsuitable during shipment or when the storage material is inadequate, loss of quality or high mortality of the predators can occur (O'Neil *et al*., 1998; Blumel and Hausdorf, 2002; van Lenteren and Tommasini, 2003; Shapiro *et al*., 2009).

Quality assessment of biological control agents has received considerable attention during the past 30 years, and the International Organization for Biological Control (IOBC) developed guidelines for quality control of many mass-produced natural enemies, including *Orius* species, in an effort to ensure that individuals produced in commercial insectaries meet minimum performance standards (van Lenteren *et al*., 2003; van Lenteren, 2009). We will use the IOBC quality control criteria to determine the effect of storage and shipment procedures used for *O.insidiosus*.

In this study, we investigated biological parameters and performance of *O. insidiosus* adults after exposure to various low temperatures and storage periods. We also assessed the influence of cold storage on the lifespan of mated and virgin females of *O. insidiosus*. We evaluated whether the quality of predators was affected by keeping individuals in containers with different dispersal materials. Finally, the effects of shipment and delivery processes were evaluated after the insects reached their final destination by measuring their survival, the proportion of males and females, and predation capacity.

Materials and methods

The experiments were conducted at the Laboratory of Biological Control of the Department of Entomology of the Federal University of Lavras, Brazil. The predator *O. insidiosus* was reared under controlled conditions (25 ± 1 °C, 70 ± 10% RH and a photophase of 12 h) in glass vials (1.7 l) with *Anagasta kuehniella* (Zeller) eggs as food and farmer's friend inflorescence (*Bidens pilosa* L.) as oviposition substrate and moisture source. The petioles of *B. pilosa* inflorescence were wrapped with wet cotton into a bouquet and inserted in glass tube containing water. These inflorescences were directly collected from wild *B. pilosa* plants growing in the field and disinfected in a 0.12% chlorine solution before offered to females in the glass vials (Bueno *et al*., 2007). Regularly, field collected *O. insidiosus* individuals from *B. pilosa* plants were added to the laboratory rearing, to insure the genetic diversity. This maintenance rearing was done according to methodology described by Bueno *et al*. (2006) and Bueno (2009).

Effect of low temperatures and length of storage on survival, daily fecundity and longevity of *O. insidiosus* adults

Effects of cold storage on *O. insidiosus* were investigated by exposing adults to different temperatures (5, 8, 10 and 12 ± 1 °C) and storage periods (2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 days). Individuals of the 5th nymphal stage obtained from the maintenance rearing were isolated in glass tubes (2 × 8 cm) to obtain males and females *O. insidiosus* of known age for use in the experiments. For each treatment (temperature-storage period combination), 300 48 h-old adults (150 males and 150 females) were individually put in glass tubes (2 × 8 cm) containing eggs of *A. kuehniella* as a food source and moistened cotton to prevent desiccation and provide free water in the form of condensation inside the glass tubes. The glass tubes were kept in different climatic chambers at the above mentioned temperatures/storage periods, with RH 70 ± 10% and constant scotophase. After each treatment the individuals were transferred to a controlled climatic room at 25 ± 1 °C, 70 ± 10% RH and 12 h photophase and adult survival (n = 150 females; n = 150 males) and female longevity (n = 10) and fecundity (n = 10) were evaluated. For assessment of fecundity, *O. insidiosus* couples were transferred to glass tubes (2 × 8 cm) containing eggs of *A. kuehniella* (food), *B. pilosa* inflorescence (substrate for oviposition) and moistened cotton. The number of eggs laid per female and the number of surviving females was measured daily until all females died. A previous study concerning reproduction of *O. insidiosus* at different temperatures (16, 19, 22, 25, 28 and 31 ± 1 °C) showed that a temperature of 25 °C was optimal (Mendes *et al*, 2005b) and, therefore, we decided to use this temperature to test reproduction characteristics in the current study as well.

The experiment was completely randomized in a factorial 4 × 10 design (four temperatures and ten storage periods), with 10 replicates per treatment. Statistical differences between treatments were assessed with a two-way ANOVA; because the data were not normally distributed they were transformed by using the formula $\sqrt{x} + 0.5$ prior to analysis. The Scott and Knott test was used to separate the means and to compare the treatments; the level of significance was 0.05.

Effect of storage period on fecundity and longevity of *O. insidiosus* mated and virgin females after the exposure to low temperature

This experiment aimed at identifying if females mated before (called mated female) or after (called virgin female) the exposure to low temperature were influenced differently by storage at 8 ± 1 °C for 10 days. Virgin females and males were obtained by keeping 5th instar nymphs of *O. insidiosus* individually in Petri dishes (5 cm) containing moistened cotton and eggs of *A. kuehniella* until adult emergence. Mated females were obtained by keeping freshly emerged males and virgin females together for a 24 h period. According to Malais and Ravensberg (2003) and Mendes *et al*. (2003) mating in *Orius* species occurs soon after reaching maturity.

Next, both mated and virgin females of 48 h-old were put individually in Petri dishes (5 cm) containing moistened cotton and eggs of *A. kuehniella.* The Petri dishes were stored for 10 days in a controlled climatic chamber at 8 ± 1 °C, 70 ± 10% RH and continuous scotophase. After storage, females were individually put in a Petri dish (5 cm) with eggs of *A. kuehniella* (food) and a *B. pilosa* inflorescence (oviposition substrate and moisture source), and kept in a controlled climatic chamber at 25 ± 1 °C, 70 ± 10% RH and a photophase of 12 h. After storage, virgin females where kept together with one freshly emerged male for a period of 24 h. Every two days the number of eggs laid was counted and the oviposition substrate and food were refreshed. The daily and total fecundity and longevity were determined for the mated and virgin females.

The experimental design was completely randomized with two treatments (virgin and mated females) and 30 replicates. Statistical differences of the influence of coldness on mated and virgin's females were assessed with a one-way ANOVA.

Survival, proportion of males and females, and fecundity of *O. insidiosus* kept in plastic containers with different dispersal materials

The dispersal materials evaluated were vermiculite + rice hulls (the coating of the seeds of *Oriza sativa* L. (1:1), pure vermiculite, folded paper towels, fine sawdust of *Pinus* sp., and coffee husks (the residue of the coffee fruit *Coffea arabica* L. which remains after drying and cleaning). These dispersal materials were selected because they are readily available, cheap, hold moisture and facilitate the distribution of the bugs in greenhouse via pouring/shaking onto plant foliage. Also they are ecologically safe because of their biodegradability. Vermiculite is one of the currently used dispersal materials used for *Orius* species by several companies.

Two hundred individuals of *O. insidiosus* (a mix of 75% adults and 25% 5th instar nymphs freshly emerged) from the maintenance rearing were placed in plastic containers (200 mL) with an open part in the lid covered with fine mesh gauze for ventilation, *A. kuehniella* eggs (food), *B. pilosa* inflorescences (oviposition substrate and moisture source) and one of the dispersal materials. Five plastic containers were placed inside a Styrofoam box (20 × 40 cm) and kept for 72 h (the time needed to transport the predators over a distance of 200 km by normal post) in a climate room at 25 ± 1 °C, 70 ± 10% RH and 12 h photophase.

We measured the proportion of males and females surviving in each plastic container (n = 200) and the fecundity of a subset of 30 females from each container, according to the guidelines for quality control recommended for *Orius* spp. by IOBC (van Lenteren *et al*., 2003; van Lenteren, 2009). To determine the reproduction capacity, females were put individually in Petri dishes (5 cm) containing *A. kuehniella* eggs (food) and *B. pilosa* inflorescence (oviposition substrate and moisture source). The daily and total number of eggs was determined for a period of 7 days. The eggs were counted on the oviposition substrate under a stereomicroscope.

The experimental design was completely randomized and consisted of five treatments (i.e. the five dispersal materials) with five replicates per treatment. Differences among the treatments were assessed with a one-way ANOVA. The Scott and Knott test was used to separate the means and compare the treatments at a level of significance of 0.05.

Survival, proportion of males and females, and predation capacity of *O. insidiosus* after shipment and delivery

This test was following the guidelines proposed by IOBC for *Orius* spp., and refers to product-control procedures (van Lenteren *et al*, 2003). Two hundred individuals (a mix of 75% adults and 25% 5th instar nymphs freshly emerged) from the maintenance rearing were placed in a plastic container (200 mL) with *A. kuehniella* eggs (food) and *B. pilosa* inflorescences (oviposition substrate and moisture source). Vermiculite + rice hulls (1:1) were added as dispersal material. Each plastic container had an opening in the lid covered with gauze to provide ventilation. Five plastic containers with 200 individuals each (total of 1000 individuals) were placed in a closed Styrofoam box (20 × 40 cm), to protect the individuals against excessive heat, cold and rough handling. No cooling was provided in the Styrofoam box during shipment. The Styrofoam box was shipped by normal postal service and eventually delivered 72 h later at the Department of Entolomogy of the Federal University of Lavras (21°14'S 45°00'W and 918 m of altitude, Minas Gerais State, Brazil). This procedure was done weekly from September to middle October, with a total of 6 shipments. In September and October it is spring time with average maximum temperatures of 27.3 °C and 27.7 °C and average minimal temperatures of 14 °C and 16 °C at this location, respectively.

The survival of individuals and the proportion of males and females alive in each plastic container (n = 200) were determined at arrival after shipment. Also the predation capacity of 30 female predators was determined after shipment by offering them 2nd instars nymphs of the thrips *F. occidentalis*, by taking 6 females from the five plastic containers. The thrips nymphs were reared on cotyledons of cotton according to Riudavets *et al*. (1993). Females of *O. insidiosus* from the plastic container were introduced individually in a Petri dish (5 cm) containing a humid disk of filter paper (5 cm) and 30 2nd instar

nymphs of *F. occidentalis* placed on a piece (3 cm) of bean pod (*Phaseolus vulgaris* L.). The Petri dishes were kept in a climate chamber at 25 ± 1 °C, 70 ± 10% RH and 12 h photophase. The number of prey consumed was measured after 24 h. The thrips nymphs were considered consumed by the predator when the rest of the exoskeleton was found or when the internal content of the nymph was partially removed.

Results

Effect of low temperatures and length of storage on survival, daily fecundity and longevity of *O. insidiosus* adults

At 8, 10 and 12 °C survival of post-storage adults was often higher than 70% for both females and males (table 1), and survival was higher than 70% at 5 °C for the storage periods from 2 to 4 days. No adults survived at 5 °C when stored for 18 and 20 days. Survival post-storage after 16 days at 5 °C was 40.3 and 41.2% of females and males, respectively (table 1), and all the individuals died a few hours after they were transferred to 25 °C (table 2).

Adult storage length and temperature clearly affected the daily fecundity and female longevity of *O. insidiosus*. Daily fecundity decreased with increasing storage period, except at 12 °C where fecundity was similar for all storage periods (table 2). An interaction of temperature and storage period on fecundity of *O. insidiosus* was found (table 2; $F = 1.742$; df = 3, 27; $P < 0.0137$). Compared to other treatments, fecundity was higher when individuals were kept for up to 10 days in storage at 8 °C (table 2; minimum 3.0 and maximum 4.4 eggs/female/day).

An interaction of temperature and storage period on the longevity of *O. insidiosus* was found ($F = 1.977$; df = 3, 27; $P < 0.0031$). Longevity was greatest at 8 °C when stored for up to 10 days (table 3; minimum 11.2 days and maximum 14 days), and was lower at other storage lengths and temperatures.

Effect of storage period on fecundity and longevity of *O. insidiosus* mated and virgin females after the exposure to low temperature

Longevity and fecundity of virgin and mated females was affected by storage at low temperatures. The longevity of the mated females after storage was signify-

Table 1. Percentage survival of *O. insidiosus* females and males after different storage periods at low temperatures, RH 70 ± 10% and continuous scotophase, n = 150 for each sex, temperature and storage period.

Storage period (days)	Storage temperature							
	5 °C		8 °C		10 °C		12 °C	
	Females	Males	Females	Males	Females	Males	Females	Males
2	90.1	91.5	97.4	9.6	99.3	98.5	98.6	96.2
4	72.8	75.4	99.3	99.4	98.6	90.1	87.6	88.3
6	63.1	60.1	90.8	96.7	86.8	95.4	85.7	81.1
8	61.4	61.4	97.4	98.5	77.3	87.2	82.2	74.8
10	60.1	58.1	88.5	83.8	76.9	75.9	81.1	72.1
12	54.2	54.5	70.3	80.4	67.6	67.6	72.6	66.3
14	50.4	50.1	75.9	78.9	62.3	62.3	70.3	65.4
16	40.3	41.2	70.2	71.5	66.8	66.8	64.1	62.3
18	-	-	70.3	73.4	60.3	55.3	62.3	61.5
20	-	-	70.4	70.3	61.4	50.4	61.2	60.1
Average	61.5	61.5	83.1	85.1	75.7	74.9	76.5	72.8

Table 2. Daily fecundity (Mean ± SE) of *O. insidiosus* females after different storage periods at low temperatures, RH 70 ± 10% and continuous scotophase, n = 30 females for each temperature and storage period. Means followed by the same letter (lower case in the column and upper case in the row) are not significantly different (Scott & Knott test at 5% probability).

Storage period (days)	Storage temperature			
	5 °C	8 °C	10 °C	12 °C
2	2.4 ± 0.63aB	4.4 ± 0.50aA	3.9 ± 0.35aA	1.2 ± 0.42aB
4	2.0 ± 0.65aB	3.9 ± 0.29aA	2.3 ± 0.48aB	1.9 ± 0.59aB
6	2.0 ± 0.57aB	3.9 ± 0.50aA	1.8 ± 0.25aB	1.4 ± 0.45aB
8	1.5 ± 0.67bB	3.0 ± 0.64aA	2.2 ± 0.68aB	1.5 ± 0.46aB
10	1.6 ± 0.57bB	4.1 ± 0.57aA	3.0 ± 0.33aB	1.0 ± 0.23aB
12	1.2 ± 0.39bA	1.3 ± 0.37bA	2.3 ± 0.97aA	1.4 ± 0.32aA
14	1.8 ± 0.63bA	2.4 ± 0.46bA	1.4 ± 0.51bA	1.6 ± 0.27aA
16	0	1.6 ± 0.41bA	1.9 ± 0.52bA	1.4 ± 0.32aA
18	0	1.7 ± 0.37bA	0.8 ± 0.36bB	1.9 ± 0.19aA
20	0	1.8 ± 0.50bA	0.4 ± 0.25bB	1.8 ± 0.53aA

Table 3. Longevity (days) (Mean ± SE) of *O. insidiosus* females after different storage periods at low temperatures, RH 70 ± 10% and continuous scotophase, n = 30 females for each temperature and storage period. Means followed by the same letter (lower case in a column, upper case in a row) are not significantly different (Scott & Knott test at 5% probability).

Storage period (days)	Storage temperature			
	5 °C	8 °C	10 °C	12 °C
2	8.2 ± 1.80aB	11.2 ± 1.15aA	10.6 ± 1.14aA	8.6 ± 1.26aB
4	6.8 ± 1.55aB	11.6 ± 1.44aA	10.6 ± 1.93aA	7.6 ± 1.45aB
6	8.4 ± 1.83aB	12.0 ± 1.42aA	8.8 ± 1.96aB	8.2 ± 1.84aB
8	8.4 ± 1.02aB	13.4 ± 1.66aA	6.6 ± 1.15aB	7.0 ± 1.71aB
10	6.6 ± 0.85aB	14.0 ± 2.51aA	7.8 ± 1.56aB	8.4 ± 1.57aB
12	6.8 ± 0.85aB	9.4 ± 1.03bA	6.8 ± 1.63aB	9.6 ± 1.40aA
14	4.8 ± 0.80aB	7.4 ± 1.19bA	4.6 ± 1.94bB	9.4 ± 0.66aA
16	0	8.8 ± 1.34bA	4.0 ± 0.84bB	9.4 ± 0.40aA
18	0	8.6 ± 1.33bA	4.2 ±1.46bB	6.4 ± 2.26aA
20	0	9.0 ± 1.58bA	3.8 ± 1.31bB	5.0 ± 2.33aB

Table 4. Percentage survival, fraction females and total and daily fecundity (Mean ± SE) of *O. insidiosus* kept for 72 hours in plastic containers with different dispersal materials. Means followed by the same letter in a column are not significantly different (Scott & Knott test at 5% probability).

Dispersal material	Survival (%)	Fraction females	Total fecundity (eggs/female)	Daily fecundity (eggs/female/day)
Vermiculite + rice hulls	85.7 ± 2.20a	0.53 ± 0.01a	55.9 ± 1.80a	7.9 ± 0.25a
Vermiculite	55.6 ± 1.52b	0.52 ± 0.01a	46.1 ± 3.51b	6.6 ± 0.50b
Folded paper towel	51.1 ± 0.74c	0.53 ± 0.01a	39.5 ± 5.52c	5.6 ± 0.50c
Sawdust	49.5 ± 0.70c	0.53 ± 0.01a	41.0 ± 1.70c	5.8 ± 0.24c
Coffee husk	50.0 ± 1.33c	0.51 ± 0.01a	29.8 ± 1.69d	4.2 ± 0.24d

cantly higher (17.3 ± 0.63 days) than that of virgin females (14.6 ± 0.69 days) ($F = 1.543$; df = 1, 120; $P < 0.001$). About 26.8% of the virgin females did not lay eggs after 10 days of storage at 8 °C, while only 4.3% of the mated females did not lay eggs. After storage, mated females showed a higher daily fecundity (4.8 ± 0.24 eggs/female/day) and total fecundity (82.8 ± 4.48 eggs/female) than virgin females (2.8 ± 0.34 eggs/female/day and 46.9 ± 6.61 eggs/female) ($F = 2.210$; df = 1, 120; $P < 0.0001$).

Survival, proportion of males and females, and fecundity of *O. insidiosus* kept in plastic containers with different dispersal materials

The type of dispersal material significantly affected the survival of *O. insidiosus* ($F = 119.0$, df = 4, 20, $P < 0.0001$). Survival was highest (86%) with the dispersal material vermiculite + rice hulls (table 4). The proportion of males and females after storage with the different dispersal materials was similar (table 4).

The highest total fecundity (55.9 eggs/female) ($F = 9.089$; df = 4, 95; $P < 0.0001$) and daily fecundity (7.9 eggs/female/day) ($F = 9.087$; df = 4, 95; $P < 0.0001$) was found for females kept in vermiculite + rice hulls compared to females kept with the other dispersal materials. The total (29.8 eggs/female) and daily fecundity (4.2 eggs/female/day) was lowest when females were kept with coffee husks and were in between and similar when the other materials were used (table 4).

Survival, proportion of males and females, and predation capacity of *O. insidiosus* after shipment and delivery

Survival and predation capacity of *O. insidiosus* kept in plastic containers with vermiculite + rice hulls inside Styrofoam boxes after shipment and delivery was high (> 93%, table 5) and apparently not affected by a 72 h transportation period. Predators consumed an average of 15.6 nymphs (range 15.2 - 16) of western flower thrips *F. occidentalis* during 24 h (table 5). The proportion of females and males present in the plastic container was on average 0.51 (range 0.50 - 0.52).

Discussion

The ability to store entomophagous insects without quality loss is a key factor in the successful use of augmentative biological control, as it allows increased flexibility in mass rearing and transport, and it facilitates sharing of colonies of high quality between the laboratories (Etzel and Legner, 1999; van Lenteren, 2003; Bolckmans and Belda, 2008). Storage at low temperatures has proved to be a valuable tool in mass production of insects and their delivery to the release site. Coudron *et al*. (2007) reported cold-storage could assist in accumulating sufficient numbers of insects for inundative releases, for minimizing costs of retaining a colony between inoculative releases, and for off-season demands.

Table 5. Percentage survival, predation capacity (Mean ± SE) and fraction females of *O. insidiosus* after a 72 h handling, shipment and delivery period.

Sample number	Survival (%)	Average number of thrips nymphs consumed per 24 h	Fraction females
1	93.2 ± 0.91	16.0 ± 0.44	0.52
2	93.5 ± 0.43	15.2 ± 0.39	0.51
3	94.9 ± 0.30	15.8 ± 0.42	0.51
4	95.6 ± 0.28	15.4 ± 0.37	0.52
5	94.3 ± 0.43	15.6 ± 0.38	0.50
6	94.3 ± 0.34	15.8 ± 0.41	0.51
Average	94.3 ± 0.24	15.6 ± 0.16	0.51

Also for successful mass rearing and distribution of species of *Orius*, the development of reliable storage, shipment and transport processes of adults are essential elements. This study shows that *O. insidiosus* adults can be stored for up to 10 days at 8 °C, without reducing their fecundity and longevity. Van Lenteren and Tommasini (2003) reported that, in general, species of predators can be stored for short periods at temperatures between 4 and 15 °C. According to the Association of Natural Biocontrol Producers (Leppla *et al*., 2002) *O. insidiosus* can be maintained for few days at 10 °C, but that survival and oviposition decrease with increasing storage time at low temperature. The effects of cold storage can be considerable and various factors should be taken into account to reveal possible reduced post-storage quality (Leopold, 1998). Humidity levels during storage may play a role in survival for example for predatory mites (Morewood, 1993). For *O. insidiosus,* van den Meiraker (1999) reports that some source of moisture is probably essential for nymph survival, but also mentions that a study to compare the effects of absence and presence of a moisture source has not been made. High relative humidity levels are important for egg survival for the few bugs for which data are available (Richards and Schmidt, 1996; Riis *et al*., 2005). According to Malais and Ravensberg (2003), temperature and food supply are the main factors influencing the reproduction and development of *Orius* spp., and relative humidity is less important. Therefore, we only assessed temperature and food availability as main factors affecting survival during the cold storage process.

No adults of *O. insidiosus* survived when stored for more than 18 days at 5 °C in this study, and when stored for 16 days or shorter at 5 °C all individuals died within a few hours after they were transferred to 25 °C. This effect may have been due to the difference of 20 °C between the storage temperature (5 °C) and the post-storage temperature (25 °C). Due to the generally low survival of adults at 5 °C, this temperature is considered unsuitable for storage of *O. insidiosus* adults. Colinet and Boivin (2011) reported that mortality represents the ultimate level of a range of sub-lethal perturbations accumulating during chilling. Reduction of fitness-related traits in surviving individuals can be observed immediately after storage, later in development or even in the next generations. According to Colinet and Boivin (2011), cold tolerance is thus a very plastic trait that may be influenced by a range of endogenous and exogenous factors experienced before, during, or even after the cold exposure.

Mendes *et al*. (2005a) reported a temperature threshold of 12.5 °C for immature development of female *O. insidiosus*. In this study the lowest fecundities and longevities of female *O. insidiosus* were observed at temperatures of 5, 10 and 12 °C. Probably this was due to the prolonged exposition of newly-emerged female to temperatures well below or very close to the temperature threshold for development. Also the consumption of energy reserves, particularly lipids, during cold exposure apparently translate into fitness costs on reproduction and/or survival (Colinet and Boivin, 2011). Denlinger and Lee (1998) reported that the reproductive system may be vulnerable to low temperature injury and that a reduced lifetime fecundity could be the combined result of a shorter life span of the female and a reduction in the daily eggs production. Leopold (1998) suggested that prolonged exposure to low temperatures induces oxidative stress in cells.

The IOBC quality control guideline for *Orius* spp. specifies that females should produce a minimum of 2.1 eggs/female/day over a 14-day test period (≥ 30 eggs/female in 14 days) (van Lenteren *et al*., 2003; van Lenteren, 2009). In our test, the daily fecundity (4.1 eggs per day) and longevity (14 days) of the predator *O. insidiosus* post-storage for 10 days was highest at 8 °C. The fecundity found in this study is in accordance with the IOBC standard, and also with values (daily fecundity of 5.6 eggs/female at 25 °C) found by Mendes *et al*. (2005b) when *O. insidious* was not exposed to storage conditions. An important conclusion of our study is that storage of adults of *O. insidiosus* up to 10 days at 8 °C offers the opportunity for insectaries to reduce food demands of and oviposition substrate for adult predators, without significant loss of quality and with a decrease of rearing costs.

In addition to identification of the optimal storage conditions, we found a very interesting effect related to the mating conditions of the predator: pre-storage mated *O. insidiosus* females showed a much higher total fecundity (82.8 eggs) than post-storage mated females (46.9 eggs) when stored for up to 10 days at 8 °C. Kobayashi and Osakabe (2009) found that the overwintering success of mated females was higher than that of virgin females, and suggested that the possible mechanism which may increase the overwintering success of *Orius* females after pre-winter copulation is the dona-

tion of nutrients from males through copulation. The second explanation might be that copulation affects the diapause syndrome (e.g. by accumulation of the lipid content) and/or behavioral changes to ensure insemination before overwintering. According to Lundgren (2011), the potential fecundity of predatory Heteroptera is largely tied to the number of oocytes that are initially present in the ovarioles. The realized fecundity is an extremely plastic characteristic of a female, and is strongly influenced by the physiological state of the mother during her life and the environmental conditions. Elkassabany *et al*. (1996) failed to recover males of *O. insidiosus* from the field during the late winter and early spring. Overwintering females apparently are inseminated before winter, and are fully capable to reproduce in spring. *O. insidiosus* overwinters as an adult in reproductive diapause (Ruberson *et al*., 1998).

Another factor contributing to effective storage is the storage and dispersal material used. Many of the commercially available predators are generalists and exhibit cannibalism, particularly when kept at high densities (van Lenteren and Tommasini, 2003). Cannibalism was also observed in several *Orius* species (van den Meiracker, 1999; Bueno, 2009, Bueno and van Lenteren, 2012). Mortality was low in this study when vermiculite + rice hulls was used. This dispersal material provides good support and shelter to the predator, leads to better conditions for survival and reproduction, and apparently largely prevents cannibalism. According to Bolckmans and Belda (2008), inert dispersal material allows natural enemies to avoid cannibalism. Coll and Guershon (2002) reported that omnivorous feeding habits may reduce the risk of cannibalism and allow predators to sustain themselves on non-prey food when prey is scarce without increasing the risk of cannibalism. Calixto *et. al.* (2013) found that pollen alone as food substantially increased the longevity of *O. insidiosus*. In this study the prey (*A. kuehniella* eggs) and pollen (from the *B. pilosa* inflorescence), together with the dispersal material may have largely prevented canibalism, which was also observed for *O. laevigatus* by Leon-Beck and Coll (2007). In our study the fecundity of the predator with the dispersal material vermiculite + rice hulls was higher than found for the same predator by and Tommasini *et al*. (2004) and Carvalho *et al*. (2005) who both worked under laboratory conditions with fresh females and did not use any shipment material. Coffee husks and fine sawdust may have contained chemical compounds causing reduced survival of *O. insidiosus*. Husks of *C. arabica* have a high content of phenolic compounds in addition to tannins and lignins (Ramirez-Matinez, 1988). Sawdust of *Pinus* is known to contain specific oils, resins, tannins and pigments (Morais *et al*., 2005). In contrast, vermiculite is considered inert material (Ugarte *et al*., 2008) and rice hulls consist of complex lignocellulose material (Saha *et al*., 2005). With folded paper towels as dispersal material, we hypothesize that this material did not provide enough hiding spots, as we saw that most of the predators kept walking on the wall of the plastic container. This kind of "stress" behaviour may have led to low survival in combination with cannibalism.

The percentage of living individuals of *Orius majusculus* (Reuter) and *O. laevigatus* did not reach 50%, and ranged between 68 and 84%, when wheat bran was used as shipment material (Blumel and Hausdorf, 2002). Bolkmans (2003) reported that, depending of the biological control agent and the duration of transportation and delivery, natural enemies are shipped with 5 to 15% more individuals than indicated on the container label to compensate for mortality which may occur during shipment. In our experiment, shipment by post during 72 h with vermiculite + rice hulls, *A. kuehniella* eggs and farmer's friend inflorescences, packed in plastic containers and put in Styrofoam boxes did not negatively affect the survival and predation capacity of *O. insidiosus*. The mortality of around 5% we found implies that adding about 10% more predators to the containers would compensate for shipment mortality. The predation capacity after transportation of predators was 15.6 nymphs of *F. occidentalis* per 24 h. This predation rate was similar to that of *O. insidiosus* (Calixto *et al*. 2013), *O. majusculus* and *O. laevigatus* (Monserrat *et al*., 2000; Bonte and De Clercq, 2010) and *O. sauteri* (Nagai and Yano, 1999) under no-storage conditions. The assessment of predation capacity in small arenas might not be representative for predation under natural conditions, but it is an important indicator for the quality of commercialized biological control agents.

The proportion of males and females found in the dispersal material experiments and in the plastic containers after shipment and delivery procedure were all higher than 50% females and are thus in accordance with the IOBC guideline of at least 45% females for quality control of *Orius* species (van Lenteren *et al*., 2003; van Lenteren, 2009).

In conclusion, our results lead to the following important findings which may help to economize the mass rearing and release of *O. insidiosus*: (1) the predator can be stored up to 10 days at 8 °C without loss of quality, (2) storage of mated female predators results in a much higher post-storage fecundity than storage of virgin females, (3) vermiculite + rice hulls is by far the best dispersal material of the 5 dispersal materials tested, and (4) shipment by post during 72 h in plastic containers with vermiculite + rice hulls, *A. kuehniella* eggs as food and *B. pilosa* inflorescences as oviposition substrate leads to good survival and did not decrease the predation capacity of *O. insidiosus*.

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Authors' addresses: Vanda Helena Paes BUENO (corresponding author: vhpbueno@den.ufla.br), Departamento de Entomologia, Universidade Federal de Lavras, Caixa Postal 3037, 37.200-000 Lavras, MG, Brazil; Lívia Mendes CARVALHO (livia@epamig.br), Empresa de Pesquisa Agropecuária Minas Gerais, 36.301-360, São João Del Rei, MG, Brazil; Joop C. VAN LENTEREN (Joop.vanLenteren@wur.nl) Laboratory of Entomology, Wageningen University, PO Box 8031, 6700 EH, Wageningen, The Netherlands.

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