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Quality assessment of *Riptortus pedestris* (Hemiptera: Alydidae) eggs cold-stored at different temperature and relative humidity regime

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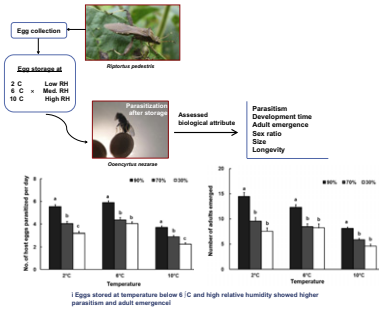
HIGHLIGHTS

- Quality of host eggs cold-stored at nine different combinations of temperature and relative humidity assessed.
- Eggs stored at 6 °C or lower together with high relative humidity had lower rate of weight reduction.
- Eggs stored at 6 °C or lower together with high relative humidity had higher parasitism and adult emergence by *Ooencyrtus nezarae*.

GRAPHICAL ABSTRACT

Eggs stored at temperature below 6 °C and high relative humidity showed higher parasitism and adult emergence.



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ABSTRACT

Supplementation of host resource can be more economical method for the biological control of insect pest compared to direct release of adult parasitoids. Periodical release of non-viable cold-stored eggs of *Riptortus pedestris* (Fabricius) (Hemiptera: Alydidae) has been found to enhance parasitism of this pest in soybean fields. To find the optimum environmental conditions for cold storage of these host eggs, we evaluated nine different combinations of temperature (2, 6, and 10 °C) and relative humidity (high 90–95%, medium 70–75%, and low 30–35%). After 30 d of cold-storage, eggs were weighed and held at 26.6 °C and 75% relative humidity for 8 d before testing. To test the eggs' suitability as hosts following cold storage, females of *Ooencyrtus nezarae* Ishii (Hymenoptera: Encyrtidae) were released individually onto batches of eggs, and parasitization rates and the development, emergence, sex ratio, adult longevity, and size of parasitoid progeny were examined. Eggs stored at high relative humidity showed less weight loss than those stored at low relative humidity. The number of eggs parasitized was highest (5.9/15) on eggs stored at 6 °C and high relative humidity. Developmental times and adult emergence were optimal on host eggs stored at 2 °C and high relative humidity. A significantly lower proportion of eggs produced male parasitoids when eggs were stored at 2 or 6 °C. Adult longevity was not affected by egg storage conditions, but adult size of progeny decreased in eggs stored at 10 °C. In conclusion, eggs of *R. pedestris* stored below 6 °C and with a high relative humidity maintained the best quality for parasitization by *O. nezarae*.

1. Introduction

Riptortus pedestris (Fabricius) (Hemiptera: Alydidae) is a polyphagous and one of the major pests of leguminous crops, especially soybean (Kono, 1989; Son et al., 2000; Kang et al.,

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2003). *Ooencyrtus nezarae* Ishii (Hymenoptera: Encyrtidae) is a gregarious, polyphagous egg parasitoid of *R. pedestris* showing non-destructive host-feeding (Hirose et al., 1996; Son et al., 2000; Paik et al., 2007; Aung et al., 2012). The parasitoid *O. nezarae* show high dispersal ability (Hirose et al., 1996) and high field parasitism on *R. pedestris* eggs in Korea (Mainali and Lim, 2012) and Japan (Noda, 1989). While augmentative release of the parasitoid to control *R. pedestris* can be an alternative control measure, it is not economical (Lim and Mahmoud, 2009) due to both the cost of rearing (Colinet and Boivin, 2011) and the fact that biological control agents have a relatively short life-span, making the production of agents shortly before their intended use important (Colinet and Boivin, 2011). However, producing or obtaining large quantities of suitable hosts right when they are needed is often difficult to achieve (Orr, 1988). Cold storage of biological control agents or the required host stage has proven to be an important method of increasing the shelf-life of natural enemies and synchronizing their supply with critical periods of pest outbreaks (McDonald and Kok, 1990; Venkatesan et al., 2000). The storage of host eggs in a state suitable for successful development of an endoparasitoid is important both for the mass rearing of natural enemies and to build up the population of natural enemies in the field by mass releasing host eggs when hosts (in the form of natural host eggs) is limiting in the field (Chen and Leopold, 2007). Various studies have found that cold-stored host eggs of various bugs, including *R. pedestris* could be successfully parasitized by egg parasitoids (Popov, 1974; Corrêa-Ferreira and Moscardi, 1993; Mahmoud and Lim, 2007), and cold-stored host eggs of *R. pedestris* have been released to increase field parasitism (Lim and Mahmoud, 2009; Alim and Lim, 2011).

The suitability of cold-stored host eggs is often evaluated on the basis of parasitism by parasitoids and the successful development of their progeny (Drooz and Weems, 1982; Kivan and Kilic, 2005). However, cold-stored eggs can lose water as they age, and parasitism of such eggs may decrease with time (Kivan and Kilic, 2005; Boivin, 2010), making the use of storage conditions able to preserve the quality of host eggs important.

Several studies have reported the effects of storage temperature, ultraviolet light, and packaging systems on the shelf-life of eggs used as hosts by parasitoids (Voegele et al., 1974; Ramos and Jimenez, 1993; Jalali et al., 2007; Alim and Lim, 2009, 2010), but only a few have investigated the effect of humidity on the cold storage of parasitoids or their host stages (Waggoner et al., 1997; Lacey et al., 1999). No studies have examined the interactive effects of temperature and relative humidity on host egg storage. Here, we assessed the optimum temperature and relative humidity for cold storage of *R. pedestris* eggs for parasitization by *O. nezarae*. We hypothesized that low storage temperature and high relative humidity would be better for extending quality of host egg for longer period of post refrigeration.

2. Materials and methods

2.1. Host egg production

Adult *R. pedestris* were collected from Andong, Republic of Korea in 2005 and maintained in the laboratory according to the method described by Alim and Lim (2009). Ascorbic acid dissolved in water (2 g/L) and soybean seeds were provided to sustain the adult *R. pedestris*, and pieces of gauze were placed in the cages as oviposition substrates. Eggs were collected every 2 d for experimental purposes.

2.2. Parasitoid rearing

O. nezarae collected from Andong was maintained on the *R. pedestris* eggs in the laboratory. Adult parasitoids 15–20 in a batch (male:female = 1:3) were placed in plastic centrifugal tubes (50 ml) for breeding. Tubes were provided with a streak of honey and a piece of moistened cotton, and were held in an incubator at 26.6 ± 0.1 °C, 31.4 ± 0.6% relative humidity, and a 16:8 h L:D photoperiod.

2.3. Cold storage

Eggs collected every 2 d from adult *R. pedestris* were immediately placed in desiccators maintained at either high (90–95%), medium (70–75%), or low (30–35%) relative humidity. Relative humidity in the desiccators was maintained using saturated salt solutions as described in Winston and Bates (1960). Salts used were $MgCl_2{\cdot}6H_2O$ for 30–35% RH, NaCl for 70–75% RH, and K_2SO_4 for 90–95% RH. Each desiccator was then placed inside an incubator maintained at 2, 6, or 10 °C. Temperature and relative humidity were recorded by using a Hobo data logger (H14-001, Onset Computer Corporation, Bourne, MA).

After 30 d of cold storage, host eggs were moved to another desiccator maintained at 75% relative humidity using a saturated solution of NaCl. This desiccator was held in an incubator at 26.6 °C, and a 16:8 h L:D photoperiod to simulate field conditions.

2.4. Weight loss and viability of cold-stored eggs

Weights of 10 batches of host eggs (10 eggs/batch) were measured on a microbalance (e = 1 mg, d = 0.01/0.1 mg; GH-252, A&D Korea Limited, Seoul, Korea) before and after the 30 d of storage at each temperature and relative humidity regime. Relative weight loss was calculated by dividing the weight loss (initial weight before storage-weight after the 30 d of storage) by the initial weight before storage.

2.5. Effect of cold-storage of host eggs on biological attributes of O. nezarae

After 8 d of being held at 75% RH, host eggs were removed from the incubator, and batches of 15 eggs were exposed to one 4–5 day-old mated female *O. nezarae* in a petri dish for 24 h. *O. nezarae* is known to parasitize 4.6 host eggs daily in laboratory condition (Alim and Lim, 2010). Thirty replicates were used for each temperature/relative humidity combination. After 24 h, the female *O. nezarae* was removed and the host eggs were placed individually into punctured eppendorf tubes (2 ml), which were then held at 26.6 °C, 75% relative humidity, and a 16:8 h L:D photoperiod to determine the number of host eggs hatching, the number of eggs parasitized, parasitoid sex ratio, development time, and emergence. In order to measure the longevity of the parasitoid progeny, the first three adult females emerged from each treatment were collected and held individually as described above at 30 °C, 75% RH, and a 16:8 h L:D photoperiod. A streak of honey and moistened cotton were provided every 3 d, and parasitoids were transferred to new tubes whenever the tubes are unhygienic. Numbers of dead parasitoids were recorded daily. In addition, the hind tibia lengths of 30 randomly selected adult female progeny were measured under a stereomicroscope using a micrometer. We did not test untreated control (i.e., viable eggs) because no significant difference in biological attributes of *O. nezarae* has been reported between viable eggs and cold-stored non-viable eggs (Alim and Lim, 2010).

2.6. Statistical analyses

The weight loss of host eggs after 30 d of cold storage, the number of eggs parasitized, parasitoid development time, sex ratio, adult longevity, hind tibia length, and adult emergence were all analyzed with two-way ANOVA, using GLM procedure in SAS and