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Effects of cold storage, rearing temperature, parasitoid age and irradiation on the performance of *Trichogramma evanescens* Westwood (Hymenoptera: Trichogrammatidae)

Abdurrahman Ayvaz[a,*], Eyüp Karasu[b], Salih Karabörklü[b], Aydln Ş. Tunçbilek[a]

[a]*Department of Biology, Faculty of Arts and Sciences, Erciyes University, 38039 Kayseri, Turkey*
[b]*School of Natural and Applied Sciences, Erciyes University, Kayseri, Turkey*

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Abstract

In this study, the effects of cold storage, rearing temperature, parasitoid age, and irradiation on the performance of the egg parasitoid *Trichogramma evanescens* were investigated. Pupae of *T. evanescens* can be stored at 4 °C for up to 3 weeks without much loss of performance. The longevity and walking speed of adults emerging from chilled pupae significantly decreased after longer storage periods. The F_1 generation of adults which emerged from pupae stored up to 3 weeks was able to parasitize as well as the control. The parasitization rate was similar at 24, 27, and 30 °C, but significantly decreased at 33 and 36 °C. Although *T. evanescens* developed to the pupal stage at 36 °C, no adult emergence was observed at this temperature. Developmental periods were longer at 24 °C than at higher temperatures. The optimum age for *T. evanescens* to successfully parasitize host eggs ranged from 24 to 90 h. The parasitization frequency of the 56–78 h aged females was higher than for the other age groups. The daily egg laying pattern of female *T. evanescens* adults was similar when they were reared on *Ephestia kuehniella* or *Plodia interpunctella* eggs. Gamma- or ultraviolet-irradiated and unirradiated host eggs were equally preferred by adult females.

Keywords: Biological control; *Trichogramma evanescens*; *Plodia interpunctella*; *Ephestia kuehniella*; Parasitoid age; Cold storage

1. Introduction

Trichogramma spp. are the most widely used natural enemies in the world, partly because they are easy to mass rear and they attack many important insect crop pests. Worldwide, over 32 million hectares of agriculture and forest have been treated annually with *Trichogramma* species for controlling insect pests (Li, 1994). Augmentative biological control by using trichogrammatid wasps offers a promising new approach for managing stored-product moths (Grieshop et al., 2006). The polyphagous egg parasitoid *Trichogramma evanescens* Westwood is commercially applied in the retail trade and the food processing industry in Germany to control stored-product moths, mainly the Indian meal moth *Plodia interpunctella* (Hübner), the Mediterranean flour moth *Ephestia kuehniella* Zeller, and the warehouse moth *Ephestia elutella* (Hübner) (Prozell and Schoeller, 1998). Control of these species using *T. evanescens* was first achieved in an industrial bakery in Germany (Prozell and Schoeller, 1998). Steidle et al. (2001) also studied the biocontrol efficacy of *Trichogramma brassicae* Bezdenko, *Trichogramma pretiosum* Riley, and *T. carverae* Oatman and Pinto against *E. kuehniella* and *Ephestia cautella* (Walker).

The egg parasitoid *Trichogramma* as a biological control agent requires that large numbers be produced by commercial insectaries. Because field requirements can vary, it is desirable to store large numbers of parasitoids to meet a fluctuating demand (Bradley et al., 2004). Storage of these parasitoids assures their availability in sufficient numbers at the time of release (Tezze and Botto, 2004). Cold storage can permit a more cost-effective production

*Corresponding author. Tel.: +90 352 437 4937x33068; fax: +90 352 437 4933.
E-mail address: ayvaza@erciyes.edu.tr (A. Ayvaz).

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schedule (Glenister and Hoffmann, 1998) providing a means to conserve biological control agents when not immediately needed (Pitcher et al., 2002). A number of *Trichogramma* species have been tested by exposing the insects to cold storage conditions (Vigil, 1971; Jalali and Singh, 1992; Piao et al., 1992; Bradley et al., 2004; Ozder and Saglam, 2004; Kumar et al., 2005). It is important to test the amenability of each *Trichogramma* species to cold since not all of them are able to be cold-stored (Tezze and Botto, 2004).

The effect of age on their ability to parasitize their host has been documented for some parasitoids (Hentz, 1998; Honda, 1998). What the parasitoid age is at the time of field release is important in obtaining a meaningful level of parasitism (Amalin et al., 2005). Temperature is one of the important factors affecting the distribution and abundance of egg parasitoids (Maceda et al., 2003). The developmental rate, fecundity, and longevity of *Trichogramma* spp. are affected by temperature (Cabello and Vargas, 1988). Knowledge of thermal requirements and the effect of temperature must be evaluated before mass rearing and release of the egg parasitoids (Maceda et al., 2003).

The effectiveness of *Trichogramma* as biological agents could be increased when used in combination with other control measures (Knipling, 1992). For control of lepidopterous pests, the combined release of sterile insects and *Trichogramma* parasitoids would be a potential control strategy. Knipling (1992) assumed that combined releases of *Trichogramma* and partially sterile moths may be practical and more effective than either technique used alone.

The objectives of this study were to determine the effects of different rearing temperatures, parasitoid ages, and irradiation of host eggs on the performance of *T. evanescens*. The effect of a range of cold storage periods on the subsequent performance of the parasitoids was also investigated to find how long they could be held in readiness for use.

2. Materials and methods

2.1. Insect rearing

Trichogramma evanescens were originally collected from eggs of *Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae) in Adana, Turkey. They were reared on ultraviolet-sterilized *E. kuehniella* eggs at 27±1 °C, 70±5% relative humidity (r.h.) with a photoperiod of 14:10 (L:D) h.

2.2. Effect of cold storage

Sixty (±5) freshly laid host eggs were glued onto pieces of cardboard with gum arabic. The cards were placed separately in glass tubes (15 × 2.5 cm) for 24 h with five female, 1-day-old adult *T. evanescens* in each at 27±1 °C, 70±5% r.h. with a photoperiod of 14:10 (L:D) h. After an 8-day incubation period, the parasitoid pupae were stored as blackened host eggs at 4 °C, 70±5% r.h. and in full darkness for up to 8 weeks. At weekly intervals after initial incubation, 20 replicates were tested at each storage period. The parasitized eggs in the glass tubes were transferred from storage to normal rearing conditions each week. Percentage adult and female emergence were determined for the parental (P) and F_1 generations. The longevity and walking speeds of adults from the stored pupae were also recorded. To assess longevity, <24-h-old females (10–20 females) were used for each treatment. The females were transferred to individual glass tubes with a drop of 15% honey/water solution as food (replaced every 48 h) and placed at 27 °C, 70% r.h. with a photoperiod of 14:10 (L:D) h. Mortality of the wasps was scored daily. Due to the reduced emergence after 4-weeks storage, a lower number of individuals were used for some treatments. When calculating walking speed, a drawing of the walking path was copied with graph paper (1 mm^2) as background. The number of 1 mm^2 crossed during 10 s of continuous walking was counted and walking speed was calculated (Steidle et al., 2001). The mean of ten 10 s periods was used to calculate the walking speed for an individual female. Ten isolated females were used for each walking speed experiment.

2.3. Effect of temperature

Twenty females (<24-h-old) were used to assess 72-h fecundity for each temperature. Each female was placed in a glass tube with an egg card bearing 50–60 *E. kuehniella* eggs and a drop of 15% honey/water solution as a food source. The glass tubes were kept at different temperatures (24, 27, 30, 33, and 36 °C). Egg cards were changed after every 24-h period. After 72 h, the wasps were removed. The number of parasitized eggs for the first 3 days, percentage adult emergence, and the sex ratio of emerging adults (% female) were determined and recorded for each replicate.

Newly emerged female adults were isolated in a glass tube. Longevity was determined by checking the number of dead females once a day, starting from adult emergence; 20 females were used at each temperature. A drop of honey/water solution was added until the wasp died and wasps drowning in honey droplets were discounted. The mean developmental time of parasitoids from egg to pupae and from egg to adult was determined at five different temperatures.

2.4. Effects of parasitoid age on parasitism

In this experiment, eight treatments were considered. Newly emerged individual females were isolated and grouped as 8, 12, 24, 54, 78, 90, 102, and 128 h of age in glass tubes and provided with freshly laid host eggs (<24-h-old) irradiated with ultraviolet light for 20 min (Mineralight Lamp, shortwave UV, 254 nm, 215–250 v, 56–60 Hz, 0.12 A). Host eggs were offered to the wasps for 24 h on egg cards prepared by spraying a fixed area with a aqueous solution of gum arabic