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Effect of cold temperature durations on the performance of the adult ***Trichogramma evanescens*** (Westwood, 1833) (Hymenoptera: Trichogrammatidae)

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Summary

In this study, the effects of cold storage on the parasitization rate, longevity, emergence rate and sex ratio of cold stored adults of ***Trichogramma evanescens*** (Westwood, 1833) (Hymenoptera: Trichogrammatidae) and their F1 progeny were investigated. The parasitoid adults were stored for 1, 2, 3 and 4 weeks at 10°C. Although parasitism rate of the cold stored adults declined depending on the increasing storage periods, parasitization per F1 female did not change. The longevity of parental adults and of its F1 progeny decreased depending on the length of the storage period, but the decrease in the parental generation was more prominent than that of the F1 progeny. The percent parasitoid emergence from the host eggs parasitized by cold stored adults was 96.55 % at the control but decreased to 85.09 % after 4 weeks storage. Female biased sex ratios were observed for all storage periods in the parental and F1 generation.

Key words: ***Trichogramma evanescens***, cold storage, parasitization rate, sex ratio, longevity

Anahtar sözcükler: ***Trichogramma evanescens***, soğukta depolama, parazitleme oranı, cinsiyet oranı, ömür

Introduction

Commercially produced species of ***Trichogramma*** attack the eggs of many lepidopteran species and have been used as biological control agents for a number of agricultural pests. The short generation time of ***Trichogramma*** wasps, and the fact that they can be reared on factitious hosts, allows these wasps to be produced

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quickly and affordably relative to other parasitoids (Li, 1994; Smith, 1996). Augmentative release of ***Trichogramma*** wasps offers a promising new approach for managing stored-product moths (Grieshop *et* al., 2006). The polyphagous egg parasitoid ***Trichogramma evanescens*** (Westwood, 1833) (Hymenoptera: Trichogrammatidae) are 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) (Lepidoptera: Pyralidae), the Mediterranean flour moth ***Ephestia kuehniella*** Zeller (Lepidoptera: Pyralidae), and the warehouse moth ***Ephestia elutella*** (Hübner) (Prozell & Schöller, 1998) (Lepidoptera: Pyralidae). Steidle *et* al. (2001) ***Trichogramma*** species (***Trichogramma brassicae*** Bezdenko, ***Trichogramma pretiosum*** Riley and ***Trichogramma carverae*** Oatman & Pinto) were assessed for their suitability as bio-control agents against ***Ephestia cautella*** (Walker) (Lepidoptera: Pyralidae) and ***E. kuehniella*** which are important pests in food manufacturing and processing facilities in Australia.

The egg parasitoid ***Trichogramma*** as a biological control agent requires that large numbers should 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 & Botto, 2004). Cold storage can permit a more cost-effective production schedule (Glenister & Hoffmann, 1998). It may also be a means to conserve biological control agents when not immediately needed (Pitcher *et* al., 2002). The variation in ***Trichogramma*** strains for cold tolerance and adaptability to low temperatures makes this a useful criterion for evaluation of candidate strains for inundative releases in cold conditions (Voegelé *et* al., 1988). A number of ***Trichogramma*** species have been tested by exposing the insects to cold storage conditions (Vigil, 1971; Jalali & Singh, 1992; Piao *et* al., 1992; Bradley *et* al., 2004; Özder & Sağlam, 2004; Kumar *et* al., 2005; Karabörklü & Ayvaz, 2007). Much of these studies report the amenability of the immature stages of ***Trichogramma*** species against cold storage. It is important to test the suitability of each particular species to cold since not all of them are able to be cold stored (Tezze & Botto, 2004).

Few studies were reported on the cold tolerance of the ***Trichogramma*** adults. The determination of cold tolerance of the adult parasitoids would be useful for some parasitoid release programs. Because, conservation of adult biological control agents would be applicable when the weather conditions changed unexpectedly during release period. We aimed to determine the amenability of ***T. evanescens*** adults to the cold and to estimate the effect of such storage on the quality of the adult parasitoid and its F1 progeny. In the current study, we have tested the tolerance of ***T. evanescens*** adults at low temperature and determined parasitization rate, longevity, emergence and sex ratio after cold storage.

Material and Methods

Insect culture

E. kuehniella stock culture was obtained from laboratory colonies at the Department of Plant Protection, Faculty of Agriculture, Ankara University. ***E. kuehniella*** larvae were reared on a mixture consisting of 1 kg wheat flour, 55 g yeast and 30 g wheat germ (Marec et al., 1999), and the rearing conditions were 27 ± 1 °C, 70 ± 5% R. H., and a photoperiod of 14:10 h (L:D). This culture was maintained under these rearing conditions for about 40 generations.

T. evanescens were originally collected from eggs of ***Ostrinia nubilalis*** Hübner (Lepidoptera: Pyralidae) sampled in Adana, Turkey. They were reared on ultraviolet sterilized ***E. kuehniella*** eggs at 27 ± 1 °C, 70 ± 5% R.H., and a photoperiod of 14:10 h (L:D). Eggs were irradiated with ultraviolet light for 20 min (Mineralight Lamp, Shortwave UV, 254 NM, 215-250 Volts, 56/60 Hz, 0.12 AMPS).

Cold storage of *Trichogramma evanescens* adults

Freshly laid ***E. kuehniella*** eggs were glued with gum Arabic (Merck, Darmstadt, Germany) on each prepared cardboard. The cards were separately put in glass vials (1.6 cm diameter x 10 cm height) for 24 h with 1-day old ***T. evanescens*** adults at rearing conditions (27 ± 1 °C, 70 ± 5% R.H. with a photoperiod of 14 h light and 10 h dark). The parasitized eggs were incubated at the rearing condition until adult emergence. The adults emerged were put into glass vials individually. The glass vials containing adult parasitoid were stored at 10 °C, 70 ± 5 % R.H. and in constant darkness at one week intervals for 4 weeks. Fifteen replicates were used for each storage period. Controls were subjected to the normal rearing conditions (27 ± 1 °C, 70 ± 5 % R.H. with a photoperiod of 14 h light and 10 h dark) and compared with cold-exposed groups.

Parasitism rate of cold stored adults and of its F1 progeny

The glass vials containing cold stored adults then were transferred to normal rearing condition. The wasps were provided egg cards consisting of fresh ***E. kuehniella*** eggs (approximately 40 ± 5 host eggs for each wasp) for 24 h. A drop of 15 % honey/water solution was supplemented as a food source. The parasitized eggs were detected by their black color indicating that the parasitoid larvae within the host egg had pupated. The number of emerged adults was also determined by counting both emerged adults and emergence holes on the host eggs. The percentage of female adults and sex ratio were estimated. The parasitism performance of the F1 progeny obtained from cold stored adults was also evaluated as in the parental generation. Fifteen replicates were used for parasitization trials of F1 progeny.

Longevity of cold stored adults and of its F1 progeny

After 24 h parasitization period ***T. evanescens*** females from every trial group were isolated in a separate glass tube. The longevity of adult females for each storage period was noted from the time adults emerged to the time of their death. A drop of honey/water solution was added until the wasp died and wasps drowning in honey droplets were discounted. The longevity was also determined of F1 progeny for each storage period as in the parental generation.

Statistical analysis

Data from the experiments were subjected to analysis of variance (ANOVA) using SPSS for Windows, version 10.0 (SPSS Inc. Chicago, IL, USA). Data were transformed using arcsine $\sqrt{x}$ transformation to meet normality, which is recommended for ANOVA (Steel & Torrie 1980). Means were separated at the 5% significance level by using the Tukey-Kramer HSD posttest.

Results and Discussion

Effect of cold storage on the parasitism rate of parental adults and of its F1 progeny

Long-term cold storage reduced the parasitism performance of parental ***T. evanescens*** (Table 1). The mean parasitization per female was significantly reduced after cold exposure depending on the length of exposure time ($F=27.19$; d.f.=4; $P<0.0001$). The mean parasitization per female was 36.26 in the control, but reduced to 21.57 in adults exposed to cold for 4 weeks. The percent adult emergence was 96.55 % at the control but decreased to 85.09 % after 4 weeks storage periods. Percentage of adult emergence from the host eggs parasitized by cold-exposed ***T. evanescens*** adults did not change up to 2 weeks period, but significant reduction was observed after 3 weeks and above storage periods ($F=35.57$; d.f.=4; $P<0.0001$) (Table 1).

Cold exposure did not change the percentage of female adults for all treatments ($F=1.06$; d.f.=4; $P>0.05$). Female biased sex ratios were observed for all storage periods ranging 1.12 to 1.78 (Table 1).

Parasitization performance of F1 progeny emerged from cold-exposed adult parasitoids was not significantly different up to 4 weeks exposure ($F=1.47$; d.f.=4; $P>0.05$). The mean parasitization of F1 progeny was ranged from 33.12 to 36.14 after 4 weeks of storage.

The emergence rate and percentage of female adults of the F1 progeny did not change depending on the cold storage periods (for adult emergence, $F=2.23$; d.f.=4; $P>0.05$, for percentage of female emergence $F=1.06$; d.f.=4; $P>0.05$) (Table 2). Female biassed sex ratios were also evident in the F1 generation ranging 2.39 for untreated control to 1.71 for 4 week storage period (Table 2).

Table 1. Parasitization performance of the cold stored parental adults of ***Trichogramma evanescens*** (Westwood) on ***Ephestia kuehniella*** Zeller at 27 °C

Storage (Week)	Parasitization per female (Mean ± SE)	Percent adult emergence (Mean ± SE)	Percentage of female (Mean ± SE)	Sex ratio (Female/Male)
Control	36.26 ± 1.26a *	96.55 ± 1.43a	64.08 ± 6.71a	1.78
1	26.06 ± 0.57b	94.28 ± 2.36a	61.05 ± 7.20a	1.56
2	22.35 ± 1.28b	98.74 ± 1.05a	62.39 ± 7.99a	1.65
3	21.63 ± 0.90c	88.91 ± 1.05b	54.28 ± 7.42a	1. 18
4	21.57 ± 0.88c	85.09 ± 1.23b	52.87 ± 8.85a	1.12

* Means followed by the same letter in a column are not significantly different at P<0.05 (ANOVA by Tukey-Kramer HSD posttest).

Table 2. Parasitization performance of the F1 progeny of cold stored ***Trichogramma evanescens*** (Westwood) adults on ***Ephestia kuehniella*** Zeller at 27 °C

Storage (Week)	Parasitization per female (Mean ± SE)	Percent adult emergence (Mean ± SE)	Percentage of female (Mean ± SE)	Sex ratio (Female/Male)
Control	34.91 ± 1.47a *	92.86 ± 1.77a	70.56 ± 7.40a	2.39
1	35.66 ± 0.90a	88.33 ± 1.30a	67.17 ± 4.25a	2.04
2	33.12 ± 0.74a	85.28 ± 0.98a	64.85 ± 4.94a	1.84
3	36.14 ± 0.75a	87.74 ± 0.72a	65.42 ± 3.77a	1.83
4	34.90 ± 0.78a	92.72 ± 0.76a	63.16 ± 7.46a	1.71

* Means followed by the same letter in a column are not significantly different at P<0.05 (ANOVA by Tukey-Kramer HSD posttest).

Effect of cold storage on the longevity of parental adults and of its F1 progeny

The longevity of parents and F1 progeny significantly decreased depending on the length of storage period (for parental F=17.47; d.f.=4; P<0.0001 and for F1 progeny, F=21.37; d.f.=4; P<0.0001) (Figure 1 and 2).

Cold storage of ***Trichogramma*** species have been of special interest in several studies (Vigil, 1971; Jalali & Singh, 1992; Özder & Sağlam, 2004; Kumar et al., 2005; Karabörklü & Ayvaz, 2007; Ayvaz et al., 2008). Most of these studies assessed the cold storage of eggs, larvae and pupae and very little consideration has been given to the cold storage of the adults. In the current study it was seen that the length of cold storage lowered the parasitoid quality of the stored adults, but the decrease in the parental generation was more prominent than that of the F1 progeny. The parasitization rate, adult emergence and longevity of cold-exposed parental females decreased when compared to control. A significant decline in parasitoid emergence from cold stored pupae was reported by Özder & Sağlam (2004) for ***Trichogramma cacoeciae*** Marchal, ***T. brassicae*** and ***T. evanescens*** after storage at 4 °C for 3 weeks. Iacob & Iacob (1972) observed a reduction in parasitism rate of ***T. evanescens*** after stored as eggs or larvae within the stored host at 9-12 °C.

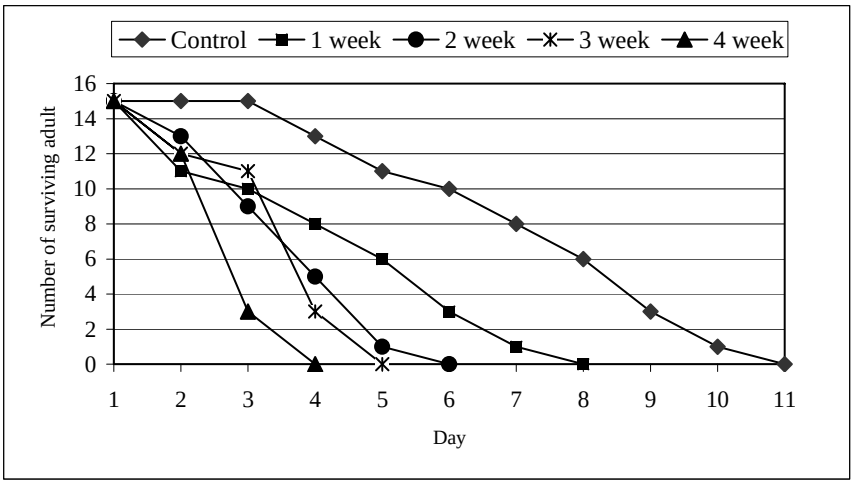

Figure 1. Longevity of the parental adults of ***Trichogramma evanescens*** (Westwood) at normal rearing condition after cold storage.

The decrease is more prominent in the parental adults than in the F1 generation. Mean longevity of parental adults was 2.0 day after stored for 4 weeks, but F1 adults lived longer (5.0 day) at the same storage period (Figure 1 and 2).

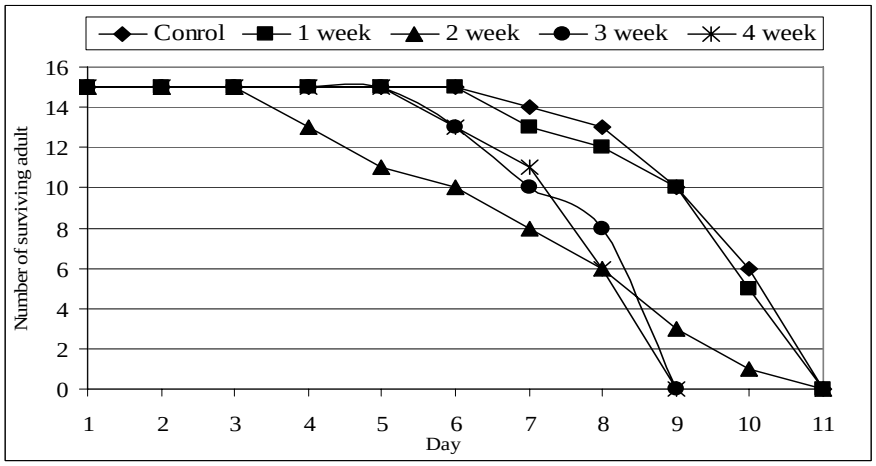

Figure 2. Longevity of the F1 progeny of the cold stored ***Trichogramma evanescens*** (Westwood) adults at normal rearing condition.

Despite the decrease in the longevity of the F1 progeny, the parasitization rate and adult emergence was not different from the control group. This showed that ***T. evanescens*** can be stored at 10 °C up to 4 weeks without much loss of its performance in the F1 progeny. In terms of the activity of F1 progeny, similar results were reported for ***Trichogramme ostriniae*** (Peng & Chen) stored at 6°C (Pitcher et al., 2002). Piao et al. (1992) found no difference in the post storage parasitism rate and progeny production for ***Trichogramme dendrolimi***

Matsumura females stored within parasitized eggs up to 30 days at 3-5 °C. Kumar et al. (2005) reported that ***Trichogramme brasiliense*** Ashmead can be stored for 10 days while ***Trichogramme chilonis*** Ishii and ***T. pretiosum*** can be stored for 20 days at 4 ± 0.5 °C without adversely affecting their emergence and parasitization efficiency.

At present study, the longevity of parental and F1 adults decreased depending on the length of storage period. Bradley et al. (2004) investigated the effect of storage temperature (4°C, 8°C, and 10°C) and time (1–8 wk) for ***T. carverae***. They showed that lower temperatures (<10°C) and 3 weeks storage had a negative impact on emergence and longevity. Jalali & Singh (1992) reported that longevity of ***Trichogramme achaea*** Nagaraja & Nagarakati, ***T. chilonis***, ***Trichogramme japonicum*** Ashmead and ***Trichogrammatoidea eldanae*** Wiggiani, declined after storage for 2 weeks at 2 and 5 °C and after 3 weeks at 10 °C.

With the hymenopteran parasitoids, survival during and following cold storage generally favours the females regardless of the stage of development that was stored (Archer & Eikenbary, 1973; Hofsvang & Hågvar, 1977; Kovalenkov & Kozlova, 1981; Jackson, 1986; Zhu & Zhang, 1987; Zhang, 1992; Whitaker-Deerberg et al., 1994).

The proportion of female progeny was not significantly modified when the newly-emerged adults were stored at 10 °C for up to 4 weeks. This demonstrates that cold storage of adults has no detrimental effects on the survival of F1 progeny depending on the sex. This result is very important where the number of released females is critical (Hassan et al., 1990; Tezze & Botto, 2004).

Although ***Trichogramma*** releases are usually as pupae, parasitoids can be released as adults in some biological control programs, to ensure a precise number of adult wasps in a given area (Smith, 1994). It would be useful to store the adults for some biocontrol program when synchronizing the parasitoids with pest population. However, the potential value for use of cold storage in commercial production needs to be evaluated in terms of economics. The reduction in fecundity of cold stored females may be unimportant from a mass production perspective, as large enough numbers of ***Trichogramma*** could be stored and used to seed new mass production colonies (Pitcher et al., 2002).

Özet

Soğuk şartlarda depolama süresinin *Trichogramma evanescens* **(Westwood, 1833) (Hymenoptera: Trichogrammatidae) erginleri üzerine etkisi**

Bu çalışmada, soğukta depolamanın ***Trichogramma evanescens*** (Westwood, 1833) (Hymenoptera: Trichogrammatidae) erginlerinin parazitleme oranı, ergin çıkış oranı, ömrü ve cinsiyet oranı üzerine etkileri laboratuvar koşullarında araştırılmıştır. Parazitoit erginleri 1, 2, 3 ve 4 hafta boyunca 10 ^{0}C de depolanmıştır. Soğukta depolanan erginlerin parazitleme oranları depolama süresine bağlı olarak azalma göstermesine karşın bu

erginlerden gelişen F1 erginlerinin parazitleme oranlarında önemli bir azalma gözlenmemiştir. Soğuğa maruz bırakılan erginler ve bunlardan gelişen F1 erginlerinin ömürleri, depolama süresine bağlı olarak azalmıştır; fakat ömür uzunluğundaki azalma atasal erginlerde F1 erginlerine göre daha belirgin olmuştur. Parazitlenen yumurtalardaki erginlerin çıkış oranı kontrolde % 96.55 iken, bu oran 4. hafta sonunda % 85.09'a gerilemiştir. Tüm depolama peryotlarında, soğuğa maruz bırakılan erginler ve bunlardan gelişen F1 erginlerinin cinsiyet oranı dişiler lehinde olmuştur.

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