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Effect of Short Term Cold Storage on the Quality of *Trichogramma Brassicae, T. Cacoeciae*, and *T. Evanescens* (Hymenoptera: Trichogrammatidae)

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EFFECT OF SHORT TERM COLD STORAGE ON THE QUALITY OF *TRICHOGRAMMA BRASSICAE*, *T. CACOEciae*, AND *T. EVANESCENS* (HYMENOPTERA: TRICHOGRAMMATIDAE)

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ABSTRACT

Trichogramma cacoeciae Marchall, *T. brassicae* Bezdenko and *T. evanescens* Westwood (Hymenoptera: Trichogrammatidae) could be useful in biological control programs of agricultural insect pests. The possibility of storing *Trichogramma* species at low temperatures and the effect of such storage on the quality of the parasitoids and their fecundity were studied. *Trichogramma cacoeciae, T. brassicae and T. evanescens* pupae were stored 1, 2, 3, and 4 weeks at 4 ± 1 °C in a refrigerator, 60-70% R.H. and full darkness. Parasitoid emergence was 98.80%, 99.33% and 99.60% for *T. cacoeciae*, *T. brassicae* and *T. evanescens*, respectively, after 1 week of storage. Storage at 4 ± 1 °C resulted in a significant decline in parasitoid emergence after 3 weeks. Subsequent trials focused on fitness of stored pupae in terms of percentage of parasitized eggs and longevity of females. Storage at 4 ± 1°C reduced fecundity and longevity of female parasitoids.

Egg parasitoids of the genus *Trichogramma* (Hymenoptera: Trichogrammatidae) are among the most commonly used natural enemies in the world against lepidopteran pests (Hassan 1993, King 1993, Smith 1996). They are gregarious endoparasitoids with the majority of species attacking eggs of Lepidoptera, although some also have the potential to attack dipteran and coleopteran eggs (Hoffmann et al. 1955, Mansfield and Mills 2002). Codling moth (*Cydia pomonella*, L.) (Lep., Tortricidae), grapevine moth (*Lobesia botrana*, Den. and Schiff.) (Tortricidae) and European leafrollers (*Archips rosanus*, L.) (Tortricidae) have been considered potential targets for *Trichogramma* inundation in orchard crops (Hassan 1992, Cossentine and Jensen 2000, Hommay et al. 2002).

Trichogrammatid egg parasitoids are generally used for inundative biological control, as annual releases. Storage of natural enemies assures their availability in sufficient number at the time of release. Storage techniques must ensure the availability of high quality natural enemies (Bigler 1994). Therefore, the development of storage techniques for biocontrol agents is considered of utmost importance to provide flexibility and efficiency in mass production, to synchronize a desired stage of development for peak release and to make available standardized stocks for use in research (Greco and Stilinovic 1998, Leopold 1998). Although there are numerous investigations about storage of trichogrammatid species (Jalali and Singh 1992, Greco and Stilinovic 1998, Pitcher et al. 2002, Tezze and Botto 2004), natural variation among species necessitates individual studies to determine species-specific parameters. The effect of different periods of cold storage on the quality of the stored parasitoids was evaluated by measuring the following variables: adult emergence time, adult emergence proportion, female proportion, fecundity and longevity.

Thus, the aim of this research was to determine whether *T. cacoeciae* Marchall, *T. brassicae* Bezdenko and *T. evanescens* Westwood could be stored at cold temperatures for various lengths of time without significant reductions on parasitoid quality.

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MATERIALS AND METHODS

Parasitoids originally collected from *Archips rosanus* L.(Tortricidae) eggs in Turkey were identified as *T. cacoeciae* (N. Kilinçer, University of Ankara, Faculty of Agriculture, Department of Plant Protection) and reared on *Ephestia kuehniella* Zeller (Pyralidae) since 1998. *T. brassicae* and *T. evanescens* were obtained from the Plant Protection Research Institute in Izmir and in Adana (Turkey). All *Trichogramma* species were reared on *E. kuehniella* in a climatic chamber at 25 ± 1 °C, 60-70% R.H. and L16:D8. *E. kuehniella* were reared on wheat bran at 25 ± 1 °C and 60-70% R.H.

Twenty-four hour old eggs of *E. kuehniella* were sprinkled over a fine gum film on paperboard strips, with 100 host eggs per strip. The strips were placed separately in glass vials (15 × 2.5cm) with ten newly emerged parasitoids and held for one day at 25 ± 1 °C temperatures, 60-70% R.H and 16:8h (L:D) photoperiod. After four days of parasitization (the parasitized eggs turn black as parasitoids pupated), the strips were placed separately in glass vials (15 × 2.5cm), plugged with cotton, and stored at 4 ± 1 °C, 65-70% R.H. with a complete dark photoperiod for 1, 2, 3 and 4 weeks. Parasitized eggs were removed every week for all parasitoid species and incubated for emergence of parasitoids at climatic conditions described above. The number of adult parasitoids emerging from host eggs was recorded. All of the treatments at each storage period and parasitoid species were replicated ten times.

Freshly emerged adults (< 12h old) were used for determining longevity and fecundity of the adults emerging from pupae stored at cold temperatures. The fecundity and longevity of parasitoids exposed to cold storage was determined using fresh eggs of *E. kuehniella* (< 12 h old). For each parasitoid species and for each replicate, a single fertilized and unfed female, less than 6 h after adult emergence was confined in a glass vial (1.5 cm diameter by 3.5 cm height) with an egg card that had > 50 host eggs. The inside of the vial was streaked with diluted 70% honey to provide a food source for the parasitoid. After 24 h, the egg card was removed and placed in a new vial which was incubated under laboratory conditions (25 ± 1 °C, 60-70% RH and a photoperiod of L16:D8) until emergence of the parasitoid offspring. Fresh host eggs were provided daily; the number of eggs offered was more than the maximum daily female capacity of parasitism. For each combination of parasitoids, the number of eggs parasitized was determined by counting the number of eggs which had turned black on the egg card. After hatching of progeny, all black eggs were dissected to confirm the hatching of a wasp or determine mortality based on dead *Trichogramma* pupae. As a control for this experiment, we used parasitoids which emerged from host eggs held at the laboratory conditions (25 ± 1 °C, 60-70% RH and L16:D8 light photoperiod). Each treatment for all parasitoid species and temperature regimes was replicated 10 times. The effect of time and temperature on the longevity and fecundity of parasitoids was analyzed using ANOVA. Means were compared using Tukey's Tests.

RESULTS AND DISCUSSION

Storage period had a significant effect on the emergence rate of adults of *T. cacoeciae* ($F = 18.89$, df = 4, $P < 0.05$), *T. evanescens* ($F = 23.59$, df = 4, $P < 0.05$) and *T. brassicae* ($F = 10.20$, df = 4, $P < 0.05$). Emergence rates of adults from stored hosts decreased with cold for all three parasitoid species compared to the control.

Development time from pupa to adult emergence was 5 days for all samples in the control group and 6 days for all samples in each of the treatments. These results are similar to those for *T. nerudai* Pintureau and Gerding (Tezze and Botto 2004). While cold storage had little affect on development time it, affected the proportion of emerged adults as well as their quality.

Emergence rates of *T. cacoeciae* which had been stored at 4 ± 1 °C for 0, 1, 2, 3 and 4 weeks were found to be 99.40%, 98.80%, 98.20%, 85.00% and 73.80%, respectively. Similarly, emergence rates for *T. evanescens* were 99.80%, 99.60% 98.20%, 89.60% and 85.60%, respectively for the same storage conditions. *T brassicae* emergence rates were found to be 100%, 99.33%, 99.20%, 85.80% and 85.40%, respectively for the same storage conditions (Table 1). Emergence rates decreased with increasing cold storage for *T. cacoeciae* (F = 18.89, df = 4, P < 0.05), *T. evanescens* (F = 23.59, df = 4, P < 0.05) and *T. brassicae* (F = 10.20, df = 4, P < 0.05).

A reduction in the emergence due to the cold storage was also seen in other *Trichogramma* spp. by Jalali and Singh (1992), Pitcher et al. (2002). Emergence rates for *T. cacoeciae*, *T. evanescens,* and *T. brassicae* stored for 4 weeks at 4 ± 1°C were similar to rates obtained for *T. nerudai* stored for 25 days at 4 °C (Tezze and Botto 2004), but higher than rates determined by Jalalý and Singh (1992) for T. *chilonis* Ishii and *T. japonicum* Ashmead stored at 5 °C for 28 days.

Longevity of emerging adult parasitoids decreased significantly with increasing storage times for *T. evanescens* (F = 7.03, df = 4, P < 0.05) in comparison to the control (Table 1). This is similar to results found for *T. ostriniae* Pang and Chen by Pitcher et al. (2002). However, storage period was not found to affect longevity of *T. achaeae* Nagaraja and Nagarkatti and *T. chilonis* by Jalali and Singh (1992). Likewise, duration of cold storage resulted in a significant decrease in fecundity for *T. cacoeciae* (F = 15.77, df = 4, P < 0.05), *T. evanescens* (F = 27.86,df = 4, P < 0.05), and *T. brassicae* (F = 3.63, df = 4, P < 0.05) (Table 1). This

Table 1. Mean (± SE) emergence rates, longevity and fecundity of T*richogramma cacoeciae, T. brassicae and T. evanescens* held in cold storage for various lengths of time (N=10 replicates).

Storage period (Weeks)	Emergence rates (%)	Female longevity (days)	Female fecundity (number of eggs)
Trichogramma cacoeciae			
Control	99.40 ± 1.34 a	15.60 ± 1.14 a	65.33 ± 2.52 a
1 week	98.80 ± 0.84 a	15.25 ± 1.71 a	50.20 ± 1.92 a
2 weeks	98.20 ± 1.09 a	15.25 ± 10.81 a	49.20 ± 1.93 a
3 weeks	85.00 ± 3.32 b	10.80 ± 8.61 a	47.00 ± 8.92 a
4 weeks	73.80 ± 12.49 c	5.75 ± 2.22 a	13.33 ± 19.65 b
Trichogramma evanescens			
Control	99.80 ± 0.45 a	21.25 ± 3.86 a	76.20 ± 15.07 a
1 week	99.60 ± 0.55 a	16.25 ± 5.91 ab	64.66 ± 1.53 a
2 weeks	98.20 ± 1.30 a	14.00 ± 9.84 ab	58.33 ± 5.13 a
3 weeks	89.60 ± 5.55 b	7.00 ± 4.97 b	30.75 ± 2.22 b
4 weeks	85.60 ± 3.58 b	4.60 ± 1.52 b	12.00 ± 10.00 b
Trichogramma brassicae			
Control	100 ± 0.00 a	17.50 ± 9.85 a	74.67 ± 20.65 a
1 week	99.33 ± 1.15 a	16.67 ± 11.67 a	67.00 ± 22.60 a
2 weeks	99.20 ± 0.84 a	14.00 ± 7.94 a	49.20 ± 16.16 ab
3 weeks	85.80 ± 8.79 b	9.40 ± 8.68 a	32.40 ± 37.32 ab
4 weeks	85.40 ± 6.53 b	4.80 ± 4.09 a	8.33 ± 10.12 b

Within columns, the same letters indicate no significant differences between treatments Tukey's multiple comparison test, (P < 0.05).

result agrees with results for *T. ostriniae* (Pitcher et al. 2002) and for *T. nerudai* (Tezze and Botto 2004). Others have found that fecundity by *T. achaeae*, *T. eldanae* Viggiani, *T. chilonis* ,and *T. japonicum* declined rapidly after storage for 14 days at 2 ºC (Jalalý and Singh 1992).

According to this study, *T. cacoeciae*, *T. evanescens,* and *T. brassicae* pupae are amenable to short term cold storage. Longer storage periods caused a significant decrease in percent emergence, longevity and fecundity of parasitoid species. Storage of *T. cacoeciae*, *T. evanescens* and *T. brassicae* pupae at low temperature for short time could be useful for inundative biological control strategies. Moreover, since the quality of F1 longevity and fecundity were not affected, this storage technique could also be useful in release programs since these species are already adapted to the local environmental conditions.

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