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Impact of Cold Storage Temperature and Period on Performance of *Trichogramma Evanescens* Westwood (Hymenoptera: Trichogrammatidae)

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Abstract: *Trichogramma evanescens* Westwood (Hymenoptera: Trichogrammatidae) was mass reared in the eggs of *Sitotroga cereallela* Olivier (Lepidoptera: Gelechiidae). The impact of cold stored pupae of *T. evanescenes* at 4, 7 and 10C for 1 to 8 weeks was considered as adults emergence, parasitism and females' percent was considered. The results showed that these two factors of storage temperatures and time substantially and negatively influenced produced adults and F1. These effects were regressed for time at each studied temperature. Results revealed correlation values of -0.913 to -0.986 for adults and -0.880 to -0.973 for F1 with P values of 0.0006 to 0.0001, for studied characters, respectively. This reduction in stored pupae performance should be considered in *T. evanescens* mass production manipulation for biological control.

Key word: *Trichogramma evanescens*, cold storage, mass production, biological control.

INTRODUCTION

Naturally occurring biocontrol agents can play an important role in suppressing pest populations in fruit orchards, field crops and forest. Amongst these *Trichogramma* species are the most widely used insect natural enemy in the world (Li and Ying, 1994). They can easily be mass reared and they attack many important crop insect pest. Nine species of *Trichogramma* are reared in private or government owned insectaries around the world and released annually on an estimated 80 million acres of agricultural crops and forests in 30 countries (Olkowski and Zhang, 1990 and Li and Ying, 1994).

The polyphagous egg parasitoid *Trichogramma evanescens* Westwood (Hymenoptera: Trichogramidae) 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) (Lepidoptera: Pyralidae), (Prozell and Schöller, 1998).

Storage of natural enemies assures their availability in sufficient number at the time of release. 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 standard stocks for use in research (Greenberg, 1996 and Leopold, 1998).

Developing effective methods to store parasitoids without changing their fitness is a crucial step in the mass production process (Leopold, 1998). Long term storage can permit for build up of sufficient number of parasitoids for mass release as needed. Two main storage technique involving cold storage have bee used in mass rearing of *Trichogramma* species with and without previous diapause induction (Greenberg *et al*., 1996). Storage techniques involving diapause induction requires a time for induction and a time for breaking this physiological state. Depending on the time the insects should be stored and their sensitivity to diapause induction, one or the other technique can be selected.

Immatures of several *Trichogramma* species can enter diapauses or quiescence within host eggs, allowing it to tolerate long periods of subfreezing temperatures (Smith, 1996). Quiescence is an immediate response to unfavorable environmental conditions (e.g low temperature) and results in slowed or haltered development and resumes once favorable condition exist while, diapauses entails metabolic changes of organism that are not necessarily resulting in development and resumes on return of suitable conditions. Diapauses can be induced in some species of *Trichogramma* so that storage of the parasitoid is possible over long periods of time

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(Knutson, 1998). Research on *T. evanescens* also showed that diapause allowed prolonged storage of the parasitoids (Bonnemaison, 1972).

Thus, the aim of the study was to determine amenability and the optimal conditions for cold storage of *T. evanescens*.

MATERIALS AND METHODS

I. Parasitoid Rearing Technique:

Used colony of *T. evanescens* was originated from Biological Control Laboratory at Agriculture Faculty, Cairo University, Egypt. Stock cultures of a strain of *T. evanscense* were maintained on *Sitotroga cereallela* Olivier eggs at Plant Protection Laboratory of Ismailia Agricultural Research Station.

Similar sizes of white paper cards (10-2.5cm) were prepared. A thin layer of non-toxic glue was applied on the cards. Freshly laid lepidopteran host eggs of *S. cereallela* were distributed on these cards. Each card holding approximately 50 egg masses placed onto glass vials (3-8 cm).

As the adult parasitoid were emerged from the host eggs, these cards were exposed to the adult female of *T. evanescens* maintained colony to allow parasitisation for 24 h that were incubated about six days for indication of parasitism. Parasitism is relatively easy to discern that determined based on the parasitized eggs become blackened. The glass tubes were plugged with cotton wads with small droplets of pure honey bee on the inner walls to feed the wasps. The parasitism was performed under standard laboratory rearing conditions; 26 ± 1ºC, 65 ± 5% relative humidity and a photoperiod of 16: 8 hours (L:D).

II. Experimental Procedures:

The parasitized eggs reached pupal stage were used because it showed better tolerance to cold storage compared to other embryonic stage in several *Trichogramma* spp. (Jalali and Singh, 1990) Experiments were carried out in three different incubators at three different temperatures setting at 4, 7 and 10C for eight different storage periods (1, 2, 3, 4, 5, 6, 7 and 8 weeks) at 65 ± 5% RH. For each storage treatment, five glass tubes (replicates) each contained one batch 50 fully parasitized *S. cerealella* eggs were randomly assigned. Once the storage period was over, the treated tubes in each treatment were transferred to the standard rearing conditions to monitor adult parasitoids emergence.

The effect of cold storage factors (temperature x period) on the performance of the stored parasitoid *T. evanescens* was evaluated by measuring the following variables for each treatment. Adult emergence was calculated as percent of emerged adult to the total number of parasitized eggs. Parasitism as percent of parasitized eggs to total number of egg exposed. Percent females as the number of female to total number of individuals. Sex of emerged parasitoid was determined based on differences in antenna type in both sexes. The F1 progeny were also evaluated as a part of the quality assessment at standard rearing conditions. To obtain the F1 progeny, five female and five males were randomly chosen from the same treatment. A pair of female and male parasitoids was selected in glass tube (3x8 cm) for mating and then removed from the tubes after 24 h. Each female was placed in glass tube (3x8 cm) containing approximately 50 fresh *S. cerealella* eggs glued on white paper cards for 24 h. The parasitized eggs were incubated until emergence of adult parasitoids. Percent emergence, percent parasitism and percent females of F1 progeny was calculated for each treatment.

III. Statistical Analysis:

The results were analyzed using Proc Reg in SAS (SAS Institute 1998).

RESULTS AND DISCUSSION

The effect of cold storage at 4, 7 and 10C for 1 to 8 weeks on produced adults' performance of *T. evanescens* from stored pupae and F1 progeny in the eggs of *S. cereallela* was studied. Produced adults and F1 performance was considered as emergence, parasitism and females' percentage. These effects were regressed for time at each studied temperature as $Y = a + b * \text{Time}$. Obtained results are illustrated in Figs. 1 to 6. The results showed that these two factors of storage (i.e. temperature and time) substantially and negatively influenced produced adults performance and the following F1.

This model revealed correlation values of -0.913 to -0.986 for produced adults and -0.880 to -0.973 for F1 with P values of 0.0006 to 0.0001 for studied characters, respectively (Table 1).

Table 1: Constants values of regression lines.

Stage	Character	°C	r	a	b	P
Adults	Emergence	4	-0.959	100.263	-8.593	0.0001
		7	-0.986	98.138	-9.148	0.0001
		10	-0.943	107.512	-10.705	0.0001
	Parasitism	4	-0.978	91.854	-5.313	0.0001
		7	-0.997	92.401	-10.738	0.0001
		10	-0.991	88.449	-6.989	0.0001
	Percent Females	4	-0.913	78.409	-5.108	0.0006
		7	-0.965	80.268	-7.755	0.0001
		10	-0.981	73.632	-5.671	0.0001
F1	Emergence	4	-0.972	95.708	-5.272	0.0001
		7	-0.973	90.282	-9.850	0.0001
		10	-0.967	91.912	-8.431	0.0001
	Parasitism	4	-0.962	96.592	-5.951	0.0001
		7	-0.996	94.678	-7.329	0.0001
		10	-0.971	93.044	-6.326	0.0001
	Percent Females	4	-0.880	77.493	-3.223	0.0017
		7	-0.952	79.013	-6.853	0.0001
		10	-0.957	70.607	-3.660	0.0001

The Effect of Cold Storage on Adults' Emergence:

This relation is illustrated at Fig. (1). Emergence of adults was negatively affected with this treatment regardless the temperature used. The obtained slopes increased as temperature decreased. The correlation values ranged between -0.943 and -0.986 with P value at 0.0001 for all tested temperatures (Table 1).

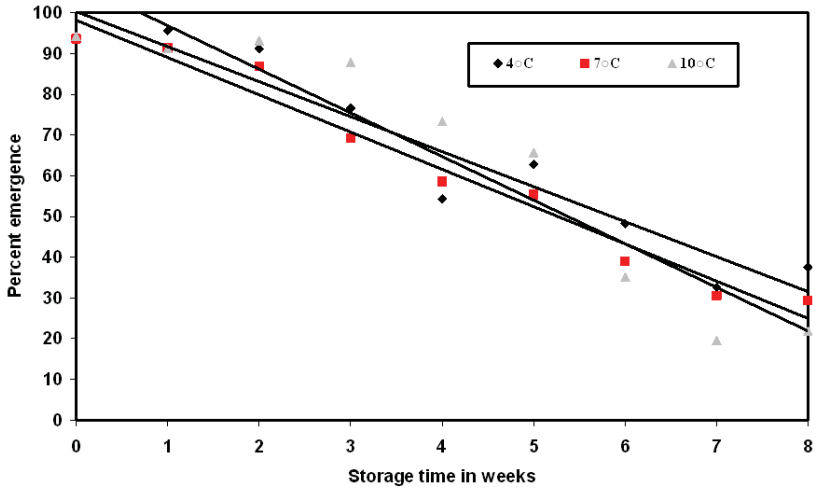

Fig. 1: Relation between pupal cold storage period and used temperature on adults emergence.

The Effect of Cold Storage on F1 Adults' Emergence:

This relation is illustrated at Fig. (2). Emergence of adults was negatively affected with this treatment regardless the temperature used. The obtained slopes were at least at 4ºC as -5.108 and maximum at 7ºC as -9.850. The correlation values ranged between -0.962 and -0.967 with P value at 0.0001 for all tested temperatures (Table 1).

The Effect of Cold Storage on Adults' Parasitism:

This relation is illustrated at Fig. (3). Percent parasitism of adults was negatively affected with this treatment regardless the temperature used. The obtained slopes were at least at 4ºC as -5.313 and maximum at 7ºC as -10.738. The correlation values ranged between -0.978 and-0.991with P value at 0.0001 for all tested temperatures (Table 1).

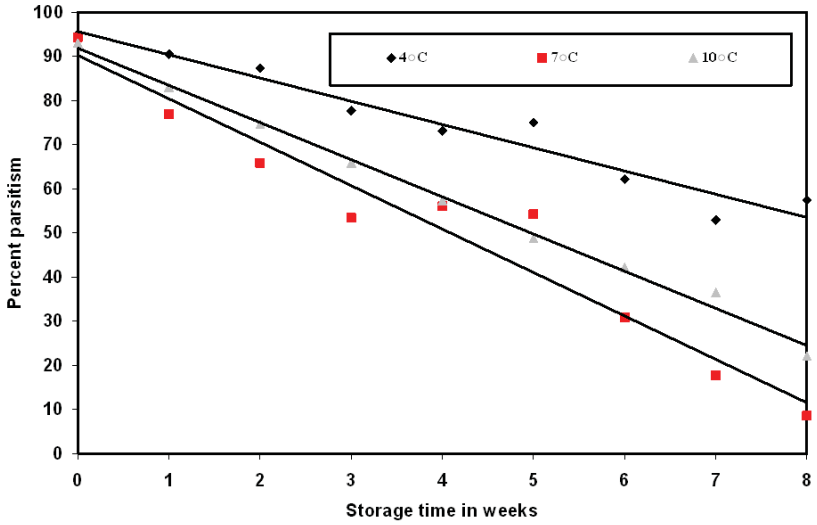

Fig. 2: Relation between adults pupal cold storage period and used temperature on F1 adults emergence.

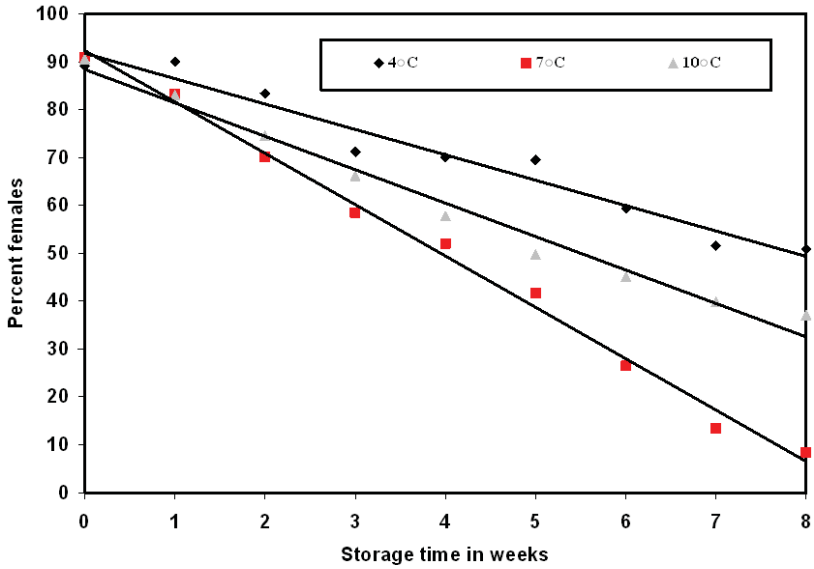

Fig. 3: Relation between pupal cold storage period and used temperature on percent prasitism.

The Effect of Cold Storage on F1 Adults' Parasitism:

This relation is illustrated at Fig. 4. Percent parasitism of adults was negatively affected with this treatment regardless the temperature used. The obtained slopes were at least at 4ºC as -5.951 and maximum at 7ºC as -7.329. The correlation values ranged between -0.962 and -0.996 with P value at 0.0001 for all tested temperatures (Table 1).

The Effect of Cold Storage on Adults' Percent Females:

This relation is illustrated at Fig. 5. Percent produced females were negatively affected with this treatment regardless the temperature used. The obtained slopes were at least at 4ºC as -5.9108 and maximum at 7ºC as -7.755. The correlation values ranged between -0.913 and -0.981 with P value ranged between 0.0006 and 0.0001 for all tested temperatures (Table 1).

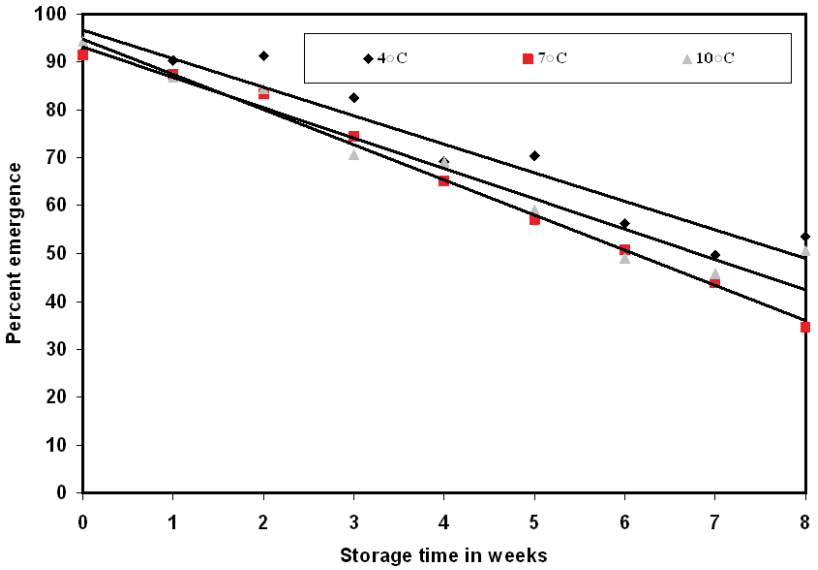

Fig. 4: Relation between adults pupal cold storage period and used temperature on F1 percent parasitism.

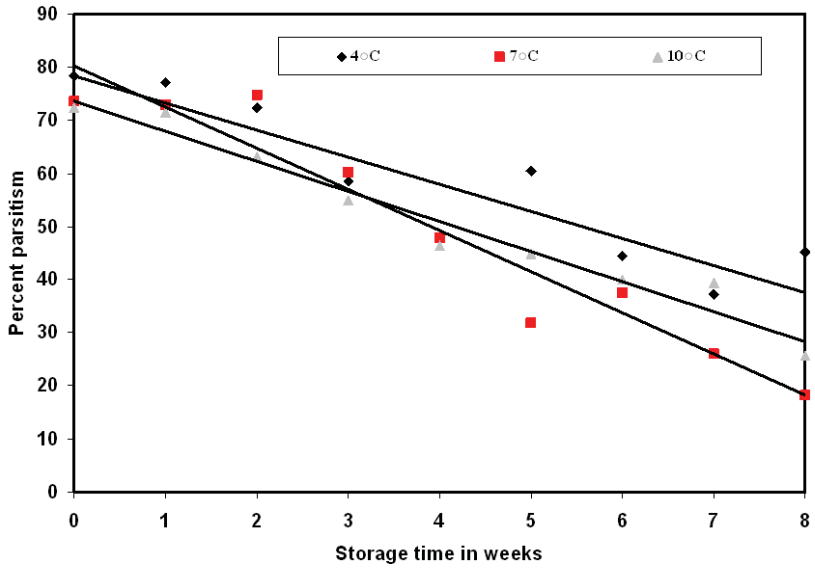

Fig. 5: Relation between pupal cold storage period and used temperature on percent femals.

The Effect of Cold Storage on F1 Adults' Percent Females:

This relation is illustrated at Fig. 6. Percent produced females were negatively affected with this treatment regardless the temperature used. The obtained slopes were at least at 4ºC as -3.223 and maximum at 7 ºC as -6.853. The correlation values ranged between -0.880 and -0.957 with P value ranged between 0.0017 and 0.0001 for all tested temperatures (Table 1).

Generally it seems that temperature of 4ºC was the least harmful tested temperature followed by 10ºC while 7ºC was the most harmful one.

The effect of temperature was nonlinear where the damage was greater at 7ºC than other two tested temperatures (i.e. higher negative slopes). The effect of both time and temperature was fitted to first degree for time and second degree for temperature of polynomial within studied range as $Y = a + b_1 * Time + b_2 * Temp + b_3 * Temp^2$ (Table 2).

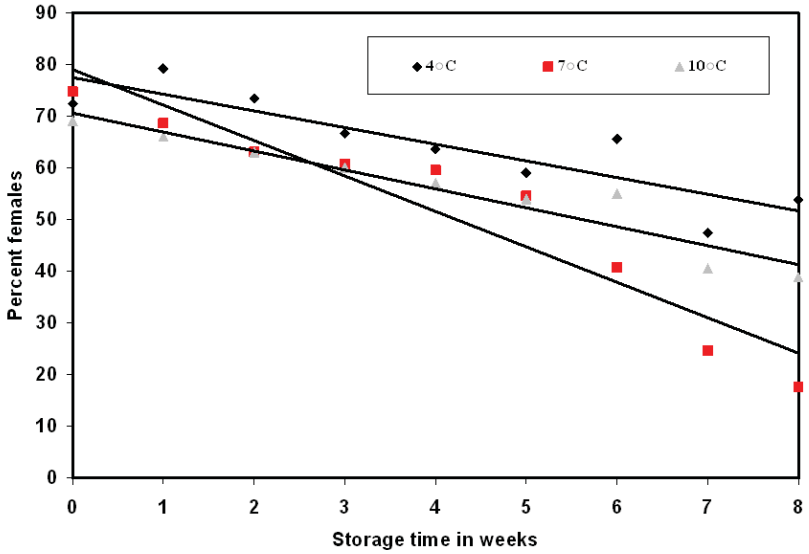

Fig. 6: Relation between adults pupal cold storage period and used temperature on F1 percent females.

Table 2: Calculated constants for the suggested models for stored pupae and F1.

Character	a	Temp		Temp ^{2}		Weeks		F value	P	R^2
		b_1	P	b_2	P	b_3	P			
				Produced adults						
Emergency	121.26	-6.025	0.2595	0.416	0.2717	-9.482	0.0001	81.78	0.0001	0.914
Parasitism	179.62	-26.730	0.0001	1.789	0.0001	-7.680	0.0001	88.03	0.0001	0.920
Percent Females	110.56	-9.285	0.0286	0.580	0.0513	-6.178	0.0001	62.66	0.0001	0.891
				F1						
Emergency	185.97	-26.884	0.0001	1.725	0.0001	-7.851	0.0001	95.30	0.0001	0.926
Parasitism	124.08	-8.466	0.0046	0.545	0.0091	-6.536	0.0001	149.34	0.0001	0.951
Percent Females	127.26	-14.945	0.0019	0.965	0.004	-4.579	0.0001	34.29	0.0001	0.817

This model revealed R^2 values of 0.914, 0.920 and 0.891 with P value of 0.0001 for produced adults emergence, parasitism and females percentage, respectively. The relative values for F1 were 0.926, 0.951 and 8.817, respectively with P value of 0.0001 for all (Table 2).

Similar studies investigating the impact of cold storage on *Trichogramma* spp clearly demonstrated that longer times accompanied with lower temperatures adversely influenced adult emergence, parasitism and percent females (Vigil, 1971; Jalali & Singh, 1992; Pitcher *et al*., (2002); Özder & Sağlam, 2004; Tezze and Botto, 2004; Kumar *et al*., 2005; Karabörklü and Ayvaz, 2007 and Ayvaz *et al*., 2008).

The adult emergence, parasitism rate and percent females of cold-exposed parental decreased when compared to control. A significant decline in parasitoid emergence from cold stored pupae was reported by Iacob and 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. Bradley *et al*. (2004) showed that lower temperatures (<10°C) and 3 weeks storage had a negative impact on emergence and longevity of *T. carverae*. Karabörklü and Ayvaz (2007) reported that emergence, parasitization and longevity of the adults emerged of *T. evanescence* from stored host eggs decreased depending on the storage periods at 4ºC, however sex ratios of the adults were not effected by storage temperature and periods.

These results in particular can be useful to preserve the quality of the founder colony of *T. evenescens* and mas produced parasitoid prior to inundative releases. Mass production may enable use of the parasitoid. Further studies on efficiency of stored parasitoid under field conditions, storage possibilities of host egg and alternative hosts may be needed to improve rearing of the parasitoid. Rundle *et al*., (2004) examined the impact of storage factors on field performance of the egg parasitoid, *T. carverae* found that the parasitod can be stored safety for up 2 weeks under laboratory conditions but after 4 weeks storage parasitoids showed a lower parasitism capacity than the control group parasitoids in field experiment.

This reduction in stored pupae viability should be considered in *T. eveanescens* mass production manipulation for biological control. It would be useful to store the parasitoid 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.

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