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Storage possibilities of scale insect predator *Chilocorus bipustulatus* (L.) eggs at different temperatures

Abstract - Mass production and release of natural enemies are among applications related to biological control. Storage of the eggs and adults of natural enemies without depriving their characteristics is an important element for the support the release when needed in the intensive release period. In this study, possibilities have been investigated of the storage of 1, 3 and 5 day old eggs of *Chilocorus bipustulatus* (L.), one of the natural enemies of California Red Scale, *Aonidiella aurantii* (Maskell), kept at 4, 8 and 12°C for different periods. Effects of the storage at different temperatures and periods on hatching rate, rate of reaching adult stage and the sex ratio were examined.No hatching was observed on 1, 3 and 5 day old eggs of *C. bipustulatus* stored for 25 days at different temperatures except for one and 3 day old eggs stored at 12°C. The highest hatching rate (94%) was found with the 3 day old eggs kept for 5 days at 8°C. The highest rate of adult development was obtained with 5 day old eggs stored at 12°C for 5 days. The percentage of females reaching adult stage was higher than that of males.

Key words: *Chilocorus bipustulatus*, eggs, storage, survival.

INTRODUCTION

Armored scales are among the economically important pests of citrus. Several species, *Aonidiella aurantii* (Maskell), *A. citrina* Coquillett, *Aspidiotus nerii* (Bouché), *Chrysomphalus dictyospermi* (Morgan), *Lepidosaphes gloveri* (Packard), *Parlatoria pergandii* Comstock, *P. oleae* (Colvée) *Mytilococcus beckii* Newman were reported from citrus in Turkey (Uygun *et al.*, 1995b). Chemical control method is frequently applied against them. Although summer oil is more selective and less harmful to natural enemies, growers usually prefer broad spectrum pesticides. Studies on the biological control of armored scales in citrus have also been performed also in Turkey (Uygun *et al.*, 1995b, Uygun & Karaca, 1998). One of the most important natural enemies of armored scales is *Chilocorus bipustulatus* L. (Coleoptera, Coccinellidae). This predator insect is very common and effective against scale insects, especially *Aonidiella aurantii*

(Maskell), in citrus orchards in Çukurova Region (Uygun *et al.*,1987; Karaca & Uygun, 1990: Uygun *et al.*,1995a).

One of the most important elements encountered in Augmentative biological control is the storage of the natural enemies without impairing their biological functions. This, because sometimes in nature, the density of natural enemies is too low to suppress the pest. In the present study the effect of storage was investigated under different conditions of eggs of *Chilocorus bipustulatus,* on hatching rate, rate of reaching adult stage and sex ratio.

MATERIALS AND METHODS

Aspidiotus nerii was reared on *Chilocorus bipustulatus.* Mass production of *A. nerii* was conducted in climate chambers at 25°C temperature and a 16:8 L:D period. Young shoots and leaves of Blue leaf weatle infested with the scale insect were obtained from field. Uninfested potatoes were colonized with crawlers of the scale. Potatoes tubers were used for further infestation for the sake of production continuity. Adults *C. bipustulatus* were collected from the field by the Stainer (1962) method and transferred to plastic boxes 13 cm in diameter and 25 cm high, containing potatoes infested with *A. nerii. C. bipustulatus* was also reared in climate chambers at 26°C temperature and 16:8 h L:D. Polyester fibres were placed in boxes, since adults lay eggs on soft surfaces (Hatting & Samways, 1991). Following the egg laying and hatching, the adults were transferred to other production boxes and production continuity was secured by supplying of infested potatoes.

Storage at different temperature and periods of C. bipustulatus *eggs kept at 26° C for different periods*

Polyester fibres with eggs on them were transferred at 4, 8, 12°C, after they had been kept at 26°C for 1, 3 and 5 days. Eggs were kept at different temperatures for 5, 10, 15, 20 and 25 days and then again put under 26°C. The eggs were then examined daily and hatching rates were determined. Larval stages were observed daily by transferring larvae on potatoes infested with different stages of *A. nerii.* Skins of moulting individuals were removed and development periodd of preadult stages were determined. Effects of different storage conditions on the hatching rate and development periods of preadult stages were investigated. Larvae were observed up to the adult stage; adults were killed and their preparations were made according to Uygun (1981). Samples were kept for one day in media with diluted phenol. Following softening, abdomens were separated from the bodies of the insects using a pin and then transferred to a 10% KOH solution. After 12-24 hours, they were immersed in distilled water. The sex ratio was determined by removing their genital organs under stereomicroscope. In addition, effects of storage at different temperatures and periods

on adult body size was calculated by measuring length and width of adults before their preparations had been made. For each temperature and period, 50 eggs were used and 5 replications were made for each treatment. Dead and live individuals were counted.

RESULTS AND DISCUSSION

Hatching rates of *C. bipustulatus* eggs stored at 4, 8 and 12°C for 5, 10, 15, 20 and 25 days after being kept at 26°C for 1, 3 and 5 days are shown in Fig. 1. The hatching rates of one day old eggs stored at 4°C were lower than that of eggs stored at 8 and 12°C. No significant difference was noticed among the hatching rates of three day old eggs stored at different temperatures. However, considerable reduction was observed in the hatching rates of these eggs depending on the storage periods. It was noticed that the hatching rates of eggs stored at 4 and 8°C, reached zero (Fig. 1). The hatching rates of five day old eggs stored at 4°C were lower than other ones (Fig. 1). As seen in 3 day old eggs, survival rates of the eggs decreased their hatching power after the 15th day.

When the data relative to the storage periods had been evaluated, it was determined that one day old eggs lost their hatching power after 15 days storage at 4°C, while hatching was not observed at 8°C after 25 days. At 12°C, the eggs survived at different rates for all storage periods. Three day old eggs survived under all conditions except 25 days storage at 4 and 8°C. However hatching rates of the eggs generally decreased with longer storage periods. All 5 day old eggs stored at different temperatures for 20 and 25 days died. Similarly, hatching rates of 5 day old eggs decreased depending on the storage period.

When the effects of egg age, storage temperature and period of the hatching rates of the eggs were evaluated together, the best result obtained was with 3 day old eggs stored at 8°C for 5 days. Eggs with different ages stored at 4 and 8°C for 25 days produced the worst results (Fig. 1).

There are some reports concerning this subject. Osman & Selman (1993) reported that, depending on the embryological development of the eggs, the effect of the storage periods on the survival rate of the eggs is very important. In addition, it was suggested that after the eggs had completed embryological development, storage did not affect the survival rate even when the storage period did last longer. In the present study, similar data were obtained determining that the hatching rates of the eggs kept for 3 and 5 days with complete embryological development were higher than the hatching rates of one day old eggs. On the other hand, results of a study on the storage possibility of *Crysoperla carnea* (Stephens) eggs, conducted by Karut & Sekeroglu (1999), showed that hatching rates of the zero age eggs, stored for different periods, were higher than 1, 2 and 3 day old eggs stored for the same periods.

The linear regression between the hatching rates of *C. bipustulatus* eggs, stored at 4, 8 and 12°C, and storage periods (Table 1) display a significant correlation between the hatching rates and storage periods except for one day old eggs stored at 12°C.

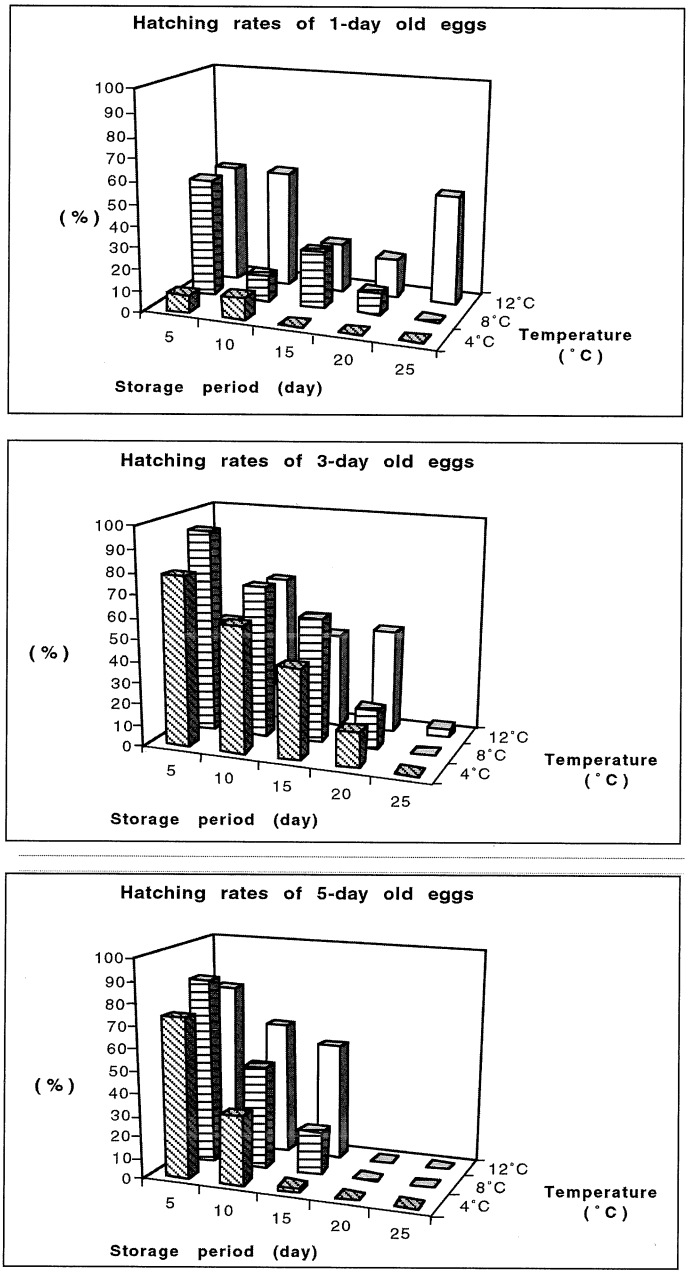

Fig. 1 - The effect of egg age, storage temperature and period on the hatching rates of the eggs of *Chilocorus bipustulatus*.

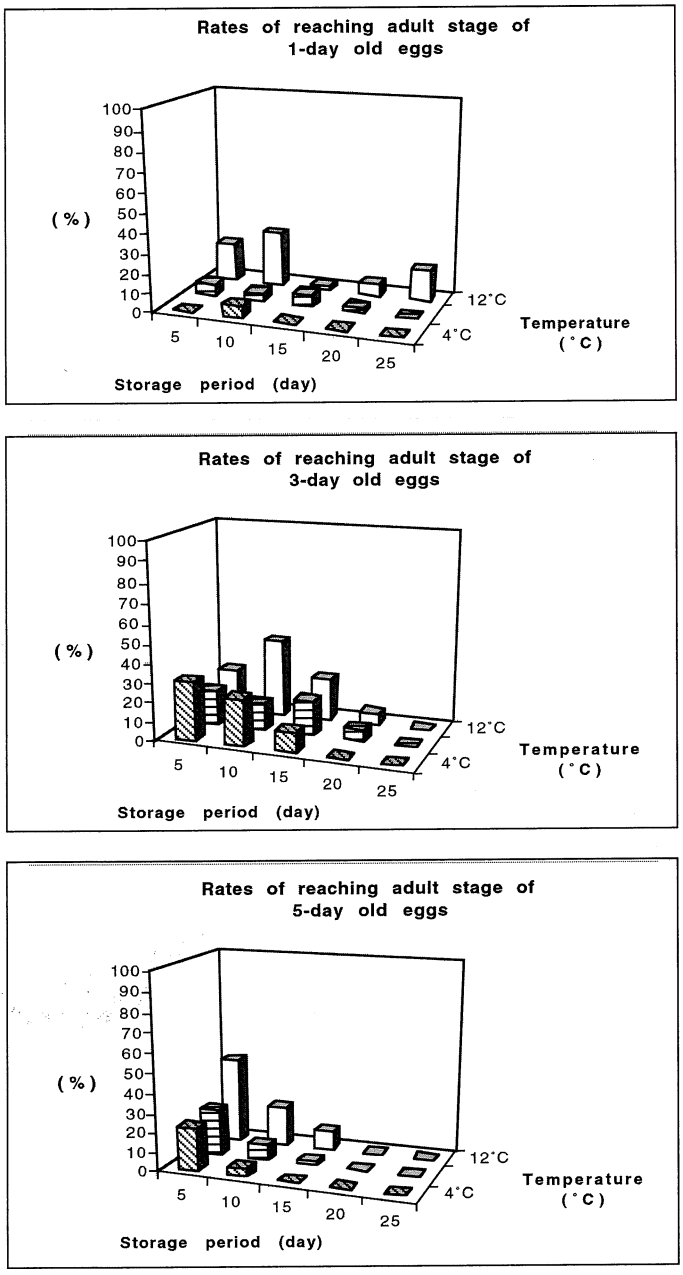

Fig. 2 - The effects of egg age, storage temperature and period on the survival of the adults of *C. bipustulatus.*

Following hatching of the eggs at different ages stored at different temperatures for different periods the development of individuals was observed up to the adult stage and the effect of these three factors on the survival of the predator was determined (Fig. 2). Individuals hatched from one day old eggs stored at 4°C for 5, 15, 20, 25 days and at 8°C for 25 days, did not reach adult stage. Among those, eggs stored at 4°C for 15, 20, 25 days and at 8°C for 25 days had not hatch already. Similarly, individuals hatched from 3 day old eggs stored at 4°C for 20 and 25 days, at 8°C for 25 days and at 12°C for 25 days and individuals hatched from 5 day old eggs stored at 4°C for 15, 20 and 25 days, at 8 and 12°C for 20 and 25 days did not reach adulthood. In this group, eggs 3 day old eggs stored at 4 and 8°C for 25 days and 5 day old eggs stored at 4, 8 and 12°C for 20 and 25 days had not hatched already. After perusal of these three graphs together, the highest rate of reaching adult stage was obtained with 5 day old eggs stored at 12°C for 5 days.

Effects of egg age, storage temperature and period on sex ratio (female %) were shown in Table 2. It was found that the rate of female individuals reaching adult stage was higher than that of males, except for 10 days storage period, with 3-day old eggs at 4°C.

Table 1 - The relationship between hatching rates of C. bipustulatus *eggs stored at 4, 8 and 12 °C and storage periods.*

Egg age (day)	The model			Coefficient of correlation		
	4 °C	8 °C	12 °C	4 °C	8 °C	12 °C
1	Y=-0.52 X + 11.4	Y=-2.20 X + 53.4	Y=-0.88 X + 52.8	0.83	0.83	0.39
3	Y=-3.96 X + 98.2	Y=-4.80 X + 120.0	Y=-2.08 X + 73.2	0.99	0.99	0.71
5	Y=-3.60 X + 75.6	Y=-4.40 X + 96.8	Y=-4.36 X + 104.2	0.88	0.95	0.95

Table 2 - Effect of egg age, storage temperature and period on sex ratio (%).

Storage Period	1-day old eggs			3-day old eggs			5-day old eggs		
	4°C	8°C	12°C	4°C	8°C	12°C	4°C	8°C	12°C
5	0.0	100.0	70.0	72.7	88.9	63.6	55.6	66.7	60.9
10	100.0	50.0	64.3	45.5	85.7	75.0	50.0	100.0	50.0
15	0.0	66.7	100.0	100.0	66.7	72.7	0.0	100.0	80.0
20	0.0	100.0	66.7	0.0	66.7	100.0	0.0	0.0	0.0
25	0.0	0.0	50.0	0.0	0.0	0.0	0.0	0.0	0.0

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