The Effects of Cold Storage on the Adult Longevity, Fecundity and Sex Ratio of *Apanteles galleriae* Wilkinson (Hym.: Braconidae)

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Abstract: The effect of cold storage on the adult longevity, fecundity and sex ratio of *Apanteles galleriae* Wilkinson, a koinobiont, solitary and early instar larval endoparasitoid of the Small Wax Moth *Achoria grisella* Fabr. was investigated. The rearing of both parasitoid and host cultures and experiments related to the effect of storing *Apanteles galleriae* adults at low temperature (+ 6°C) were conducted at 25 ± 1 °C, 60 ± 5 % RH under a photoperiod of 12:12 (L:D). Cold storage (+ 6°C) considerably influenced the adult longevity, fecundity and sex ratio of the parasitoid. Experimental evidence showed that 85.27% of adults died after a week and all the adults died within 15 days when parasitoid adults were stored at low temperature. The resistance of females to low temperatures was higher than that of males. Storage at low temperature significantly decreased adult fecundity and increased the rate of males in progeny.

Key Words: Parasitoid, Apanteles galleriae, Cold storage, Longevity, Fecundity, Sex ratio, Biological control

Apanteles galleriae Wilkinson (Hym.: Braconidae)'nin Ergin Hayat Uzunluğu, Verim ve Eşey Oranına Soğukta Tutmanın Etkileri

Özet: Koinobiont, soliter ve erken evre larva endoparazitoit *Apanteles galleriae* Wilkinson' ın ergin hayat uzunluğu, verim ve eşey oranına soğukta tutmanın etkileri konak olarak Küçük Balmumu Güvesi, *Achroia grisella* Fabr. kullanılarak incelendi. Parazitoit ve konak kültürlerinin yetiştirilmesi ve soğukta (+ 6°C) tutmanın etkileri ile ilgili deneyler, 25 ± 1°C sıcaklık,% 60 ± 5 nispi nem ve 12: 12 (A: K) fotoperiyot uygulanan laboratuar şartlarında yapıldı. Düşük sıcaklıkta tutulan parazitoitin ergin hayat uzunluğu, verimi ve eşey oranı önemli ölçüde etkilenmektedir. Düşük sıcaklıkta tutulan parazitoit erginlerinin bir hafta sonra % 85.27'si, 15 gün sonra ise tamamı ölmektedir. Dişilerin düşük sıcaklığa direnci erkeklerden daha yüksektir. Düşük sıcaklıkta tutulma ergin parazitoitlerin oğul döl verimini önemli ölçüde düşürmekte, oğul döldeki erkek oranını yükseltmektedir.

Anahtar Sözcükler: Parazitoit, Apanteles galleriae, Soğukta tutma, Hayat uzunluğu, Verim, Eşey oranı, Biyolojik kontrol

Introduction

Although adult longevity and fecundity are controlled genetically, the effect of environment is also significant. It has been determined that the developmental period and adult longevity of parasitoids in particular vary significantly according to temperature (1-5). In addition, developmental time and longevity are affected by: the type of species (6), the adult size (7, 8), mating status (mated or unmated) (7, 9), the frequency of mating in males (9), the sex of the parasitoid (2, 6, 7, 10-13), the amount and type of food consumed in the adult stage (3, 7, 11, 13-16) and, in those that complete their

development on more than one host, the type of host (17, 18).

Studies of different parasitoid species have demonstrated that adult parasitoid fecundity, sex ratio in progeny and the parasitization capacity of the female parasitoid vary according to temperature (1, 13). Additionally, fecundity, sex ratio in progeny and parasitization capacity of the female parasitoid are affected by: the age of the parasitoid (1,7, 11-13, 19-22), the host species (18, 23), the host stages (12, 20, 21), whether or not the host has been parasitized before (7, 24), the number of eggs laid by the female (1, 7, 20,

24), the time elapsed after development into the adult stage before the host is found (11, 13), the host abundance (7, 23-25), and the amount and type of food (6, 7, 20, 23). All of these show that temperature may be the most important determining factor in the mass production and application of parasitoids used for biological control.

It has been shown that *Apanteles galleriae* has the characteristics of a biological control agent (26-30). For this reason, the effect of low temperature on the adult longevity, fecundity and sex ratio of *A. galleriae* was investigated in this study.

Material and Methods

In the experiments, we used the koinobiont, solitary and early instar larval parasitoid *Apanteles galleriae* and early instar larvae of the Small Wax Moth, *Achoria grisella* as the parasitoid and host species, respectively. The rearing of the parasitoid and host cultures and the experiments to determine the effects of storing parasitoids at low temperature ($+6^{\circ}$ C) were conducted at 25 \pm 1°C, 60 \pm 5% RH under a photoperiod of 12:12 (L:D). The methods used to establish and maintain successive cultures of both the host and parasitoid species were described in a previous study (29).

First, an adequate number of adult parasitoids in a defined age group were obtained in order to determine the effects of storing adult parasitoids at low temperature on adult longevity, fecundity and sex ratio. For this purpose, a large number of pupae were taken from the parasitoid stock culture and placed in glass tubes 2 at a time. 1-3-day-old adults were taken from these tubes and 20 females and 20 males were placed together in each of six 1-liter jars containing cotton soaked with 50% honey solution. Cloth was tied around the necks of the jars in such a way that aeration was not prevented. Three of these jars were kept for 7 days and the other 3 were kept for 15 days in a refrigerator calibrated at + 6 \pm 1°C.

After 7 days' exposure to cold, the surviving adults were counted and the fecundity and sex ratio in progeny were determined using *A. grisella* as the host. Methods developed previously (30) were followed in determining the fecundity and sex ratio. No experiments were conducted with the specimens kept in cold storage for 15 days since there were no survivors at the end of this

period. The experiments were repeated three times with specimens taken from different populations at different times. All experimental results were analyzed statistically by t-test with a least significance difference of (p < 0.05).

Results

The results for fecundity and sex ratio in the progeny of A. galleriae kept for periods of 7 and 15 days at +6°C are given in the table. As can be seen in the table, the resistance of males stored in cold conditions for 7 days was lower than that of the females. After 7 days of storage, 18.33% of the female parasitoids survived, whereas only 11.11% males survived. In other words, 20 of the 180 males and 33 of the 180 females, a total of only 53 out of 360 individuals, survived. The difference between the numbers of parasitoids at the beginning of the experiment and those which survived after seven days was statistically significant, as was the difference between the number of surviving males and females (p>0.05). The weekly average fecundity of the surviving females at +6°C was 6.24 females and 15.27 males, a total of 21.51 offspring per female (Table). The sex ratios of female and male parasitoids in progeny were 29.01% and 70.99%, respectively. The ratio of males to females in progeny was considerably different (Table). No adults survived among the 360 males and females stored at +6°C at the end of 15 days.

Discussion

Temperature has a considerable effect on the life of an organism (2, 7, 13, 18, 31, 32). However, each organism requires a different temperature (2, 7, 13, 18, 31, 33). Both high and low temperatures have negative effects on living beings (2, 7, 13, 18, 32, 34). The longevity of adult *Dibrachys boarmiae* kept at +4°C for 15 days in one study was found to increase, whereas all A. galleriae adults died when they were stored at +6°C for the same length of time (7). It was reported that 92% of Lysiphlebus fabarum pupae kept at +3°C for a week developed into adults, while this figure was 48% when the pupae were stored for 2 weeks, and no adults appeared after storage for 3 weeks (32). This shows that as the duration of cold storage increases, living activity decreases and eventually stops. Our results are similar to those of other studies conducted on other species. Not only is the type of species a factor (2, 7, 13, 18, 32), but

	Duration kept in cold				Surviving Individuals		
Sex	7 (days) Surviving-Died*		15 (days) Surviving-Died		Total Fecundity*	The number of offspring / Female (Mean ± SD)*	Sex Ratio (%)
Female Male	33 a 20 b	147 a1 160 b1	-	180 180	206x 504 y	6.24±6.57 x1 15.27±16.86 y1	29.01 70.99
Total	53	307	-	360	710	21.51±23.30	

Numbers in a column or line with the same letter are not significantly different (p < 0.05).

Table:

The effect of low temperature on the adult longevity, fecundity and sex ratio of *A. galleriae*.

the stage and sex of the parasitoid and season (7, 32, 34) also play a role in change in resistance to low temperatures. The resistance of females to low temperature was higher than that of males in *A. galleriae* adults stored in cold conditions for a week (Table). That only 14.72% of *A. galleriae* adults were alive at +6°C at the end of a week means that this species has a fairly low resistance to cold. The lack of mobility in some of the surviving individuals indicates that this temperature was not suitable for the storage of *A. galleriae* adults. The greater body size of the *A. galleriae* females may be the reason why they are better able to resist cold temperatures (7).

The storage of parasitoids at low temperature considerably decreased adult longevity and fecundity. The decrease in fecundity (Table) of *A. galleriae* after storage at cold temperatures has been demonstrated in other species (32). The results obtained in the present study (Table) were lower than those of other experiments (30, 35) carried out under the same laboratory conditions

with individuals of the same species which were not stored in the cold. The female sex ratio was also very low compared to that found in other experimental results (35) in progeny obtained with no storage of parasitoids at cold temperature. When A. galleriae were stored in cold conditions (Table), the fecundity per female and the ratio of females in progeny were 21.51 and 29.01%, respectively. However, these values were 211.40 and 41.40% in one study with G. mellonella as the host, and 105.86 and 55.60% in another study with A. grisella as the host (30, 35). These experiments were carried out under the same conditions, but the parasitoids were not exposed to cold. Adult longevity, fecundity and female ratio in the progeny of parasitoids are of great importance, both in mass production and studies of biological control. Therefore, it would be beneficial to conduct further studies under different temperature, humidity and photoperiodic parameters in order to determine the proper temperature ranges for this parasitoid species, which would lead to increases in adult lifespan, fecundity and female ratio in progeny.

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