Effect of Temperature on the Immature Developmental Time and Adult Longevity of Apanteles Galleriae Wilkinson (Hym. Braconidae)

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Abstract:
The greater wax moth, Galleria mellonella (L.), is a known pest of the Egyptian and worldwide beekeeping. The solitary endoparasitoid, Apanteles galleriae Wilkinson is one of important natural enemies of the pest. The study aimed to determine the effect of temperatures under near-continuous darkness on the immature developmental time, growth of parasitized larvae and adult longevity of A. galleriae. The duration of developmental period of male was less than that of female irrespective of temperatures. At 20°C temperature and 75% RH, the duration ranged from 21.7 days for males and 23.8 days for females, decreased to 18.1 and 19.2 days at 25°C, 17.1 and 18.1 days at 27°C and 16.0 and 17.3 days at 30°C, respectively. Parasitism by A. galleriae retards the growth of G. mellonella larvae. The initial weight of wax moth larva at the time of parasitization was 3.5±0.2 mg and was significantly lower (9.9±0.7 mg) prior to parasitoid egression than that of the non-parasitized larva (89.5±5.2 mg). The parasitized host larvae eventually weighed up to 70 to 88% less than the non-parasitized larvae. The adults survived starvation, water- or one-day honey feeding for few days, while they lived for significantly longer periods when they provided daily with pure honey. Longevity of females fed on pure honey was significantly longer than males at all tested temperatures. The results may be of value for developing mass rearing techniques and biological control of wax moths by A. galleriae.

Keywords: Apanteles galleriae, temperature, immature development, adult longevity, host growth

1. Introduction
Studies on the effects of constant temperatures on the development of different hymenopteran parasites were carried out by several workers (Sullivan1965, Heron 1967, Philogenean and Benjamin 1971). Browning and Oatman (1981) reported that different constant temperatures influenced the developmental time, adult longevity and progeny production in Hyposer exiguae (Viereck) to a greater extent.

Wax moth is a pest of the Egyptian and worldwide beekeeping. The pest is persistent and causes great economic loss in Egypt. It is distributed throughout all apiaries and causes extensive damage to bees wax both within and outside the beehive. The greatest losses in Egypt occur during the dearth period or just after the end of the honey crop. After honey extraction, farmers are left with large quantities of old or damaged combs and cell cappings (Hegazi et al.2017). There are also excess drone combs that are primarily suitable for drone production and honey storage. These combs are usually destroyed by wax moth if not recycled or properly stored (Kwadha et al.2017).

Two types of wax moths exist, namely, Greater ‘larger’ Wax Moth (Galleria mellonella (L.)) and lesser ‘smaller’ Wax Moth (Achoria grisella (F.)). Both moths cause the same type of damage to combs in storage and within the hive. The level of damage is much greater within a short period when attacked by the greater wax moth. Both moths will co-exist in the same bee hive. Larvae consume the comb, particularly the honey bee brood combs. While feeding the larvae forms tunnels and silken webs insidethe comb and cause greater damage to the comb structure (Hanumanthaswamy and Rajagopal 2017).

Apanteles galleriae Wilkinson, is well known parasitoid that is present in Egypt. The wasp is a koinobiont, solitary and early instar larval endoparasitoid of lepidopterous species (G. mellonella, Achoria grisella F., Achoria innotata Walker and Vitula edmandsaePackard) (Shimamori 1987, Watanabe 1987, Whitfield et al. 2001 and GulatiandKashish (2004) that can cause significant damage to comb in honeybee hives. Studies conducted on this species have been mainly focused on
its biological characteristics and host-parasitoid interactions (Uckan and Gulel 2000, 2001; Uckan and Ergin 2002, 2003). Little is known about the interaction between this wasp and wax moth larvae (Kwadha et al. 2017). We aimed to show the effect of temperatures under near-continuous darkness on the immature developmental time, growth of parasitized larvae and adult longevity of A. galleriae.

2. Materials and Methods

2.1. Insect Cultures

Stock cultures of G. mellonellae and the parasitoid A. galleriae were established from adults collected at several beehives located in Alexandria, Egypt. Infusions of field-collected insects were made to maintain genetic diversity of the insects. The A. galleriae colony was maintained using third- and fourth-instar G. mellonellae larvae on diet developed by Kulkarni et al. (2012) and modified by Hegazi et al. (2017). Both species develop with no known diapaus, in Egypt. The host and parasitoid cultures were maintained in two separate containers. The rearing of wax moth cultures was done in transparent plastic boxes (8.5 cm x 8.5 cm x 14.5 cm), provided with artificial diet to a depth of 6-8 cm. The A. galleriae wasp was reared on the 3rd-4th instars of wax moth larvae, in the same boxes but smaller ones (7.5 cm x 5.5 cm x 5.5 cm) (see Hegazi et al. 2017). Droplets of pure honey were used as a food source for wasp adults to ensure maximum reproductive success. Rearing insects were conducted in a rearing room at 25±2°C, 75±5% relative humidity under near-continuous darkness.

2.2. Experimental Procedure

The effect of temperature on the immature-developmental time of A. galleriae was evaluated by the rearing parasitized fourth in star larvae (head capsule width, 59±0.11 mm) at four different temperatures (20, 25, 27 and 30°C), under near-continuous darkness and Relative Humidity (RH) 75±5% until host larvae produced parasitoids. Parasitization was performed by exposing the 4th wax moth larvae to A. galleriae young mated female wasps in a Petri dish (5.5 cm x 1.8 cm) provided with 0.3 cm depth diet and small corrugated papers as described by Hegazi et al. (2017). The larvae were observed until the parasitism occurred. An ovipositional attack was defined as an encounter resulting in insertion of the ovipositor into the host larva. Larvae that accidentally received more than one ovipsitional thrust were discarded. Once host larvae were stung, they were then placed in the plastic Petri dish (3 larvae/dish). In the dishes, the diet spread to a depth of 0.5 cm and small corrugated papers and used during the entire larval and pupal phases of parasitized larvae (15-20 replicate). Parasitized hosts larvae (45-60 larvae/expt.) were monitored daily and observations were made on emergence date and duration of pupal stage of the parasitoid. Parasitized host larvae of each experiment were monitored in three isolated incubators to avoid pseudo replication. The experiments were conducted in incubators (type Hann, Munden, Germany). All incubators were located in a controlled room.

To study the effect of the braconid parasitoid, A. galleriae on the growth of the wax moth larvae, the host larvae were parasitized by placing fourth in star larvae in a Petri dish with a female parasitoid and the parasitized larvae were placed on the diet following a single ovi position. Parasitized and control larvae of similar age were weighed again when 15% of parasitized host larvae start producing parasitoids, and only those larvae that produced parasitoids were included in the results.

To study the effect of supplementary foods on the longevity of A. galleriae adults, nine sets of freshly emerged wasps (0-2 h), each composed of 50 individuals were selected. Each set was distributed into 5 glass vials (2.5 x 10 cm) at rate of 5 males and 5 females per vial. Four feeding systems were checked each at 20 and 25°C. These supplementary foods were: (1) no food or water; (2) water; (3) only a meal of honey; (4) honey meal/day throughout the adult’s life. The ninth set of individuals were fed daily on honey but only at 30°C. The food was renewed daily. Mortalities were recorded daily till the death of all wasps.

3. Results

The effect of temperature on the immature developmental time of A. galleriae, a koinobiont, solitary and early in star larval-endoparasitoid of the wax moth, G. mellonella, is shown in Table 1. The results of this study showed that temperature had significant effects (for males: F=36.9; df=3, 36; P<0.05; for females: F=55.8; df=3, 36; P<0.05) on the immature developmental time of A. galleriae wasp (Fig. 1). At 20°C, the egg-larval stage period in female individual was 13.9±0.12 days decreased to 11.7±0.02, 11.6±0.04 and 11.6±0.04 days at 25, 27 and 30 days, respectively. The pupal period in female individuals decreased from 9.88±0.07 days at 20°C to 7.29±0.06, 6.87±0.09 and 6.37±0.08 days at 25, 27 and 30°C, respectively. The shortest total developmental periods of the parasitoid was observed at 30°C, where the developmental period ranged from 21.7 days for males and 23.8 days for females, decreased to 18.1 and 19.2 days at 25°C, 17.1 and 18.1 days at 27°C and 16.0 and 17.3 days at 30°C, respectively. The total duration of immature developmental period of males and females was significantly different at both of low (20°C, t=2.63, P<0.05) and high (30°C, t=3.1, P<0.05) rearing temperatures. However at 25 or 27°C, the differences between the total duration of immature developmental period of males and females were not significant (Table 1).

Parasitism by A. galleriae retards the growth of G. mellonella larvae. At 30°C, the mean initial weight of both parasitized and non-parasitized wax moth larva at the time of Parasitization was 3.5±0.2 mg (Fig. 2). Taken daily biometric measurements of parasitized larvae were not suitable for wasp development (markedly prolonged). So, only the weight of both parasitized and non-parasitized hosts were taken at the time of parasitism and at the time of wasp egression. In general, the bodyweight of parasitized larvae increased at a slower rate than that of the non-parasitized larvae, and this
resulted in significant difference prior to parasitoid egression between the mean weights of parasitized and non-parasitized larvae (at 30°C; F=956.4;df=2,27;P<0.05). The weight of parasitized larvae just prior to parasitoid egression (8-9 d after parasitism) was significantly lower (9.9±0.7 mg) than that of the non-parasitized larvae (89.5±5.2 mg) (Fig.2). The parasitized wax moth larvae eventually weighed up to 70 to 88% less than the non-parasitized larvae. The parasitized larvae leave the diet 1-2 days before parasitoid egression. Only one parasitoid emerged per single larva. During emergence, the parasitoid larva tears a hole on the host cuticle and pupated in a white silken cocoon. Most hosts died soon after the egression of the parasitoid larvae.

The average weight of fresh cocoons produced at 30°C was 3.3±0.1 mg for male and 4.3±0.1 mg for female (n=20/sex, data not shown). The difference between sexes was significant (t=5.5; P<0.05). At emergence, the wasps can be sexed by the blunt abdomen of males and long ovipositor of the females. The adult parasitoid is black in color. Adult males and females were able to mate soon after the emergence at all the temperature regimes, and the average duration of copulation ranged from 15 to 30 seconds. The wasp is meandrous in the wild but engages in polyanidry ‘multiple mating’ under excess of fresh virgin males in the small rearing cages (data on sperm counts were not taken). Males will immediately start chasing the females and successful mating can only be ascertained when females remain motionless and male and female are attached for several seconds at the abdomen. Some honey-fed wasps of 20-30 days old were able to mate with fresh emerged females. The adult parasitoid has no preoviposition period.

Longevity of A. galleriae was studied by exposing freshly emerged wasps (0-2h) to different feeding regimes as mentioned above. The longevity of male and female adults living under different feeding condition is given in Table 2. Both sexes lived longer when the adults were maintained at 20°C than those lived at 25 or 30°C. Freshly emerges adults strongly attracted to the honey droplets. The wasps might feed on pure honey until their abdomen swell out so that they could not walk for some minutes. Both sexes significantly lived longer when the adults were fed on honey (Fig.3). The lowest life span was among the fasted adults. At 20°C, the adults survived starvation for an average of 26±0.1 and 33±0.2 days for males and females, respectively. However, insignificant increase in average was found when the adults held at 20 or 25°C were fed on water (Table 2).

The honey-fed sets of the adult wasps differed significantly in their life span. They survived for few days when they were fed on one meal of honey, while a significant increase in their longevities was observed when they were fed daily on honey. The effect of temperature on daily honey-fed groups was significant (for males: F=657.3; DF=2, 27; P<0.05; for females: F=60.8; DF=2, 27; P<0.05). The mean longevity of adult male and female was 41.8±1 and 52.7±1.9 d, respectively, when were fed daily on honey at 20°C. The mean longevity of adults significantly decreased to 23.1±0.8 and 27.4±0.9 d at 25°C and to 11.6±0.6 and 21.04±1.5 d 30°C for males and females, respectively. The difference between the longevity of both sexes fed on honey was significant (at 20°C, t=8.73; at 25°C, t=7.2; at 30°C, t=13.5; P<0.05).

4. Discussion

Temperature is the most important physical factor affecting biological aspects of insect parasitoids (Butler Júnior and López 1980, Harrison et al. 1985, Noldus 1989). Detailed knowledge of the wasp’s life cycle, and how it responds to temperature, is necessary data for mass production of the parasitoid. It has been determined that the development al period and adult longevity of insect parasitoids vary significantly according to temperature (NealisandFraser1988, Hale Michael and Smith1994). The koinobiont end parasitoid, A. galleriae is the most important enemy of young greater wax moth larvae. The effect of temperature under near-continuous darkness on the immature developmental time, adult longevity of the wasp, and growth of wax moth larvae, G. mellonellae parasitized by A. galleriae was investigated. The duration of total developmental period (from egg to adult stage) of male was less than that of female irrespective of temperatures. At 20°C temperature and 75% RH, the developmental period ranged from 21.7±0.6 days for males and 23.8±0.5 days for females and decreased as the temperature increased. However, the photoperiod (Light:Dark) represents another factor that affected the immature developmental time of this non-diapausawasp (Hegazi et al.2017). They reported that at 20°C, the continuous darkness (0L:24D) accelerated development to both the egg larval and pupal stages, while both the short photo period (6L:18D) or continuous light (24L:0D) slowed down the development at the same temperature. On the other hand, Uckan and Ergin (2002) reported that the quality host diet had an effect on the developmental speed of both the host and the wasp. At 25±1°C, 60±5% RH, and a photoperiod of 12L:12D h, the period from egg to adult for both sexes of A. galleriae changed according to the diet of the host on which the parasitoid completed its immature development. Development time of females was greater than that of males on all host diet. The number of days from oviposition to first emergence of adults was 25 and 27 d for males and females, respectively, with blackened comb.

The nutritional requirements of the synovigenic parasitoid adults assume a considerable importance in biological control, e.g. increase longevity and fecundity of adult wasps (Hegazi and El-minshawy 1981 and Jeffrey et al. 2012). The longevity experiments of the present study using A. galleriae was ps indicated that feeding systems (no food; water; one meal honey or daily meal honey) significantly affected the survival period of the wasp females. In the absence of food source all wasps showed the shortest longevity. In general, parasitoid longevity under each feeding condition was food dependent. Also, adult longevity varied by sex when adults were provided with different supplementary food. The effect on temperature for daily honey-fed groups was significant. Uckan and Ergin (2002) reported that when the poorer wax moth, Achroia grisella (F.), was provided with pure honey, the mean longevity of A. galleriae of males lived a short average of 5.75 d, whereas females lived 10 d. Males lived slightly longer than females when host species provided with blackened comb. But, females lived significantly longer than males with the other types of host diet (dark yellowish and pure
The present study showed that longevity of females was significantly longer than males at all tested temperatures. On the contrary, Uckan and Gulen (2000) reported that male wasps lived longer than females.

Our results indicated that pure honey is necessary for a significant prolongation in lifespan of A. galleriae adults. Adults supplied with pure honey lived longest at 20°C. The mean longevity of adult male and female was 41.8±1 and 52.7±1.9 d, respectively, when the wasps were fed daily on honey at 20°C under near darkness. A.galleriae and its host larvae, G. mellonella normally live in near-continuous darkness in the beehives, A. mellifera. It seems that the wasp's habitat under near-continuous darkness was good for the wasps to live longer. The development of the parasitized wax moth larvae by A. galleriae was disrupted. When the parasitoid larvae finished development, the body weight so host larvae were significantly less in comparison with non-parasitized hosts. The parasitized wax moth larvae eventually weighed up to 70 to 88% less than the non-parasitized larvae. These changes in growth of parasitized wax moth larvae may be attributed to virus particles associated with the calyx fluid of the wasp female (Vinson and Iwantsch1980). Daily biometric measurements of the parasitized larvae was not suitable for wasp development, may result to pseudo-data due to markedly prolongation of caused by daily handling the host larvae. Similar study was undertaken by Wani et al. (1994) to examine the larval development and precocious pre-pupal changes of G. mellonella parasitized by A. galleriae. Our results suggest that A. galleriae has considerable potential as a biological control agent for controlling the wax moth. The present results may be of value for developing programs for mass rearing techniques for A. galleriae and biological control of wax moths by A. galleriae wasps.

5. Conclusion

The effect of temperature (20, 25, 27 and 30°C), on the immature-developmental time of A. galleriae showed that the duration of developmental period of male was less than that of female irrespective of temperatures. The total developmental periods of the parasitoid was shortest at 30°C and longest at 20°C. The parasitized host larvae eventually weighed up to 70 to 88% less than the non-parasitized larvae. The study showed that longevity of females was significantly longer than males at all tested temperatures.

6. References

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Appendix

Figure 1: Mean Duration (Days, ±SE) of Total Developmental Period of Immature Stages of Galleriae under Near Continuous Darkness and Different Constant Temperatures Mean Values with the Same Letter in the Same Sex Are Not Significantly Different Than Each Other (P > 0.05)

Figure 2: Final Mean (±SE) Weight Attained by Parasitized 4th Instar Host Larvae by A. Galleriae Compared with Unparasitized (Control) Hosts
Figure 3: Longevity (Days, ±SE) of A. Galleriae Wasps Fed on Pure Honey under Different Constant Temperatures, Bars with the Same Uppercase or Lowercase Letter Are Not Significantly Different (P < 0.05)

Table 1: Mean Duration in Days (D, ±SE) of Egg-Larval and Pupal Stages of A. Galleriae Under Different Constant Temperatures and Near Complete Darkness

| Temp. °C | Food type | Egg-larvae Stage (d) | Pupal Stage (d) | Total (d) |
|---------|-----------|----------------------|----------------|-----------|
|         |           | Male | Female | Male | Female | Male | Female | Male | Female |
| 20      | ------ (control) | 12.89±0.17 | 13.90±0.12 | 8.73±0.15 | 9.88±0.07 | 21.7±0.6 B | 23.8±0.5 A |
| 25      | Water (a) | 11.77±0.02 | 11.78±0.02 | 6.26±0.10 | 7.29±0.06 | 18.1±0.3 A | 19.2±0.4 A |
|         | Honey (b) | 11.20±0.03 | 11.60±0.04 | 5.82±0.08 | 6.87±0.09 | 17.1±0.4 A | 18.1±0.5 A |
| 30      | Honey (a) | 10.80±0.01 | 10.90±0.02 | 5.34±0.06 | 6.37±0.08 | 16.0±0.2 B | 17.3±0.3 A |

Table 2: Longevity (Days, ± SE) of A. Galleriae Fed on Various Food Types under Different Constant Temperatures and Near Complete Darkness

| Temp. °C | Food type | Length of Adult Life ±SE |
|---------|-----------|--------------------------|
|         |           | Male | Female | Male | Female | Male | Female |
| 20      | ------ (control) | 1-3 | 2.6 ± 0.1 | 2-4 | 3.3 ± 0.2 |
|         | Water (a) | 2-5 | 3.9 ± 0.3 | 3-5 | 4.4 ± 0.2 |
|         | Honey (b) | 3-4 | 4.2 ± 0.2 | 3-6 | 5.6 ± 0.2 | 33-65 | 41.8 ± 1.8 B | 35-73 | 52.7 ± 1.9 A |
| 25      | ------ (control) | 1-3 | 1.6 ± 0.2 | 2-3 | 2.3 ± 0.3 |
|         | Water (a) | 3-5 | 3.2 ± 0.1 | 3-5 | 3.4 ± 0.0 |
|         | Honey (b) | 3-5 | 4.4 ± 0.1 | 3-6 | 4.9 ± 0.3 | 8-28 | 23.1 ± 0.8 B | 19-32 | 27.4 ± 0.9 A |
| 30      | Honey (a) | 6-18 | 11.6 ± 1.4 B | 9-44 | 21.0 ± 1.5 A |

Within rows, Means followed by the same letters are not significantly different (P < 0.05).

(a) Fed for one day
(b) Fed daily