Journal of Agricultural Science and Food Technology
Vol. 3 (1), pp. 1-6, February, 2017
ISSN: 2465-7522
Full Length Research Paper
http://pearlresearchjournals.org/journals/jasft/index.html

Effects of Photoperiod on the Immature Developmental Time of *Apanteles galleriae* Wilkinson (Hymenoptera: Braconidae)

Esmat Hegazi[1*], Wedad Khafagi[2] and Neama Aamer[1]

Accepted 26 January, 2017

[1]Department of Entomology, Faculty of Agriculture, Alexandria University, Egypt.
[2]Plant Protection Research Institute, Alexandria Egypt.

ABSTRACT

***Apanteles galleriae Wilkinson* and its host larvae*, Galleria mellonella (L.)* normally live in near-continuous darkness in the beehives, *Apis mellifera*. Both species develop with no known diapaus, in Egypt. The effects of photoperiods (Light: Dark): 0L: 24D; 6L: 18D; 12L: 12 D; 18L: 6 D and 24L: 0D at constant temperatures: 20, 25, 27 and 30°C on the immature developmental time of the wasp were investigated. The influence of photoperiod on the developmental time of the egg-larval and prepupal-pupal stages of the parasitoid is significantly noticeable at 20°C. The continuous darkness (0 L: 24 D) accelerated the development of both the egg-larval and pupal stages, while both the short photoperiod (6 L: 18 D) or continuous light (24 L: 0 D) slowed down the development. At complete darkness, the number of days from oviposition to emergence of adults was 21 and 23 days for males and females, respectively, prolonged to 27 and 29 days at short photoperiod (6 L:18 D) and 31 and 33 days at long photoperiod (18L:6 D), respectively. This pronounced effect of photoperiod at 20°C on the immature developmental time of the wasp disappeared as the temperature increased to 25, 27 or 30ºC.**

Key words. *Apanteles galleriae, Galleria mellonella*, photoperiod, temperature, immature development.

*Corresponding author. E-mail:eshegazi@hotmail.com.

INTRODUCTION

Most studies on the effects of photoperiod have been primarily concerned with diapause induction in insects. Photoperiod is a major factor in determining the developmental pathway (continued development vs. diapause) of many insect pests (Eizaguirre et al., 1994; Fantinou et al., 1995). Moreover, the effect of photoperiod on other developmental characteristics has also been determined (Lopez et al., 1995; Fantinou et al., 1996). However, data in the literature on the effect of photoperiod on life history parameters of non- diapaused insects are scarce. *Apanteles galleriae* Wilkinson is a koinobiont, solitary and 4th to 5th instar larval endoparasitoid of some lepidopterous species (Shimamori, 1987; Watanabe, 1987; Robeiro, 1996; Whitfield et al.*,* 2001) that can cause significant damage to comb in honeybee hives. Singh (1962) reported that *A. galleriae* may be host specific for *Galleria mellonella*. Roberiro (1996) found that 4th and 5th instars are the preferred hosts, the female lays its eggs singly inside the caterpillar, and the life cycle takes 16 to 22 days. *A. galleriae* parasitoid and its host larvae, *G. mellonella* normally live in near-continuous darkness in the beehives or wax comb stores. These species are with no known diapause in Egypt (Kares, 1978). Studies conducted on *A. galleriae* parasitoid have been mainly focused on its biological characteristics and host parasitoid interactions (Uckan and Gulel, 2000, 2001; Uckan and Ergin, 2002, 2003). It has been shown that *A. galleriae* has the characteristics of a biological control agent (Hegazi et al., 1982; Uckan and Gulel, 2000). In Egypt, some, Beekeepers expose blackened infested beeswax combs in open area away from the bee hives in order to accommodate the *A. galleria* as a protection tool. The present study investigates the interactions between temperature and photoperiod in the larval-pupal development stages of the non-diapause*, A. galleriae*. The study may contribute to a better understanding of the evaluation of diapause of *A. galleria* in Europe.

MATERIAL AND METHODS

Insects Cultures

G. mellonella and its koinobiont, solitary larval endoparasitoid parasitoid, A. galleriae, were taken from cultures maintained in insectary of Economic Entomology Department, Alexandria University Egypt, round the year. Infusions of field-collected insects were made to reduce the negative effect of inbreeding for both cultures. G. mellonella was reared in mass culture on modification of diet developed by Kulkarni et al. (2012). The components of present diet (ca.8 kg) were: wheat flour (2.5 kg), wheat bran (2.5 kg), honey (750 g), Medical dried yeast (500 g), low fat milk powder (350 g), glycerine (800 g), methyl p-hydroxybenzoate (4 g) and freeze blackened beeswax powder (250 g). The host and parasitoid cultures were maintained in two separate containers. The rearing of wax moth cultures was done in transparent plastic boxes. The lid of the plastic container was cut in the middle in a circle form "ringed hole, 5 cm" and fine wire mesh screen was carefully glued on the surface of the ringed hole. Host culture containers (8.5 x 8.5 x14.5 cm) were provided with artificial diet to a depth of 6 to 8 cm. The wire mesh (100x 30 /sq) enables female moths to lay eggs on its surface. The wire mesh screen creates a rough surface for easy collection of eggs without injury. These eggs are later removed carefully from the surface of this wire mesh screen for further laboratory incubation or hatching. The A. galleriae wasp was reared on the 3rd to 4th instars of wax moth larvae (see below). Honey droplets containing 50% honey and 50% water were used as a food source for wasp adults.

Experimental Procedure

The effect of photoperiod on the immature -developmental time of A. galleriae was evaluated by the rearing parasitized fourth instar larvae (head capsule width ,59 ± 0.01 mm) at different temperatures (20, 25, 27 and 30°C), combined each with "L:D" 0:24, 6:18, 12:12, 18:6 and 24:0 and Relative Humidity (RH) 60±5% until host larvae produced parasitoid. The parasitoid cocoons were kept under the same condition until adult wasps emerged. Mating in A. galleriae, wasps occurs as soon as both sexes are present, thus male and female wasps held together in glass vials (25 by 100 mm) for 24 h were presumed mated. Parasitization was performed by exposing wax moth larvae to A. galleriae mated female wasps in a 60 mm diameter and 15 mm high Petri dish provided with 0.3 cm depth diet and small corrugated paper. Then, 2 to 3 mated female wasps were aspirated out of the mating vials and introduced into the Petri dishes containing 4 to 6 host larvae. Once female wasps were introduced into the oviposition Petri dish they began searching. The dish could then be partially opened and closed with relative ease without losing the parasitoids. 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 ovipositional thrust were discarded. Once host larvae were stung, they were then placed in the plastic containers (3 larvae/container).

The containers of the parasitized wax moth larvae (15 to 20 replicates/expt) were the same as above but smaller (5.5 cm x 5.5 x7.5 cm). The diet spread to a depth of 0.5 cm and used during the entire larval and pupal phases of parasitized larvae. Parasitized hosts larvae (45 to 60 larvae/expt) were monitored daily and observations were made on emergence date of parasitoid larvae and duration of pupal stage of the parasitoid. To avoid pseudoreplication, parasitized host larvae of each experiment were monitored in three isolated growth chambers "incubators", that is, the replicates of a temperature/photoperiod were exposed in three incubators.

The experiments were conducted in incubators (type Hann, Munden, Germany), equipped with eight fluorescent 30-Watt cool white flurescent tube per cabinet. In all experiments, the light onset started at 5 am. The light intensity during photophase was approximately at 1270 foot candles. Light was measured with a Weston light meter (Model) (Weston Electrical Instrument Company, Newark,NJ). Small fans were used for cooling electronic equipment, operated during the light cycle to remove heat produced by lights. All cabinets were located in a controlled room.

Statistics

Statistical analyses were conducted using SPSS 17.0. Differences in the durations of immature development between different treatments were compared using one-way analysis of variance (ANOVA) followed by multiple comparisons analysis using Tukey's HSD test. Student's t-test. SPSS 8.0 software (SPSS Inc., Chicago, IL) was used for statistical analyses.

RESULTS

The interactions between temperature and photoperiod in larval development on non-diapausing *A. galleriae*, is shown in Table 1. The results showed that at 20ºC, photoperiod had significant effects on the immature developmental time of *A. galleriae* wasp (Figure 1).The

Table 1. Duration in days (mean ±SE) of egg- larval and pupal stages of *A. galleriae* under different photoperiodic regimes.

Tempeature.℃	Developmental stage (d)	Photoperiod (L :D, h)				
		0:24	6:18	12:12	18:6	24:0
20	Egg- larva : Male	12.9±0.20	18.02±0.04	No data	18.5±0.07	No data
	Female	13.9±0.20	19.1±0.03	No data	19.8±0.04	No data
	Pupa Male	8.7±0.10	9.4±0.10	No data	12.6±0.03	No data
	Female	9.9±0.10	10.7±0.10	No data	14.9±0.1	No data
25	Egg-larva : Male	11.8±0.02	11.9±0.03	11.4±0.08	11.7±0.02	10.7±0.01
	Female	11.8±0.01	12.3±0.02	11.8±0.20	12.2±0.01	10.9±0.01
	Pupa Male	6.3±0.10	6.4±0.10	7.0±0.020	6.1±0.10	6.1±0.10
	Female	7.3±0.10	7.5±0.10	8.0±0.20	7.1±0.10	7.1±0.10
27	Egg. Larva : Male	11.2±0.03	13.04±0.05	12.04±0.04	10.8±0.03	No data
	Female	11.6±0.04	13.7±0.04	12.6±0.02	10.9±0.01	No data
	Pupa Male	5.8±0.10	5.7±0.10	5.3±0.10	5.3±0.10	- No data
	Female	6.5±0.10	6.4±0.30	6.3±0.10	6.5±0.10	No data
30	Egg-Larva : Male	10.8±0.01	9.9±0.03	No data	11.2±0.03	11.5±0.06
	Female	10.9±0.02	10.0±0.00	No data	11.6±0.01	12.04±0.05
	Pupa Male	5.3±0.10	5.2±0.20	No data	5.1±0.10	5.1±0.010
	Female	6.4±0.10	6.0±0.00	No data	5.7±0.05	5.5±0.10

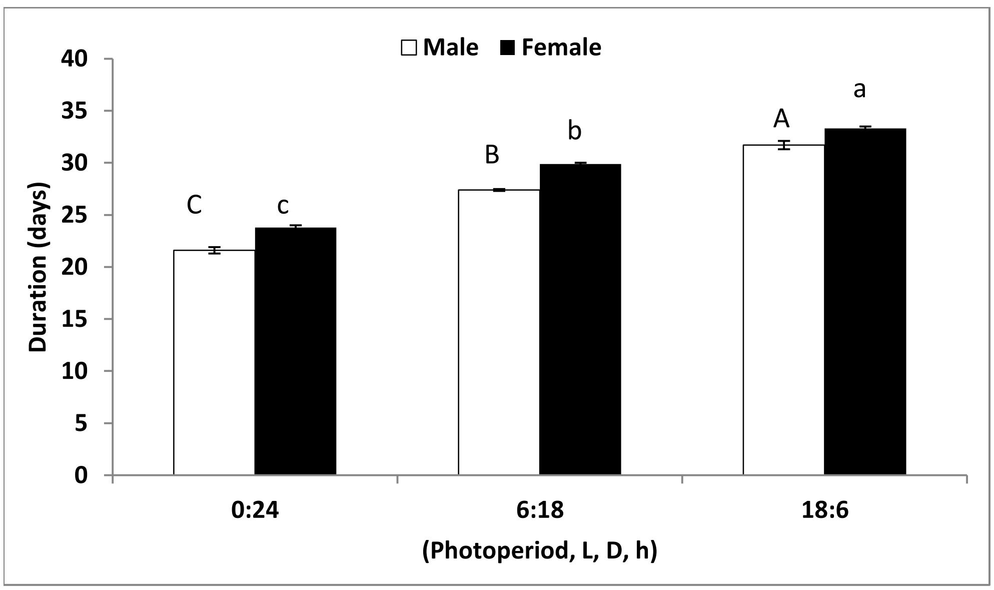

Figure 1. Duration in days (means±SE) of total developmental period of immature stages of *A. galleriae* under three photoperiodic regimes at 20±1℃. Bars with the same uppercase or lowercase letter are not significantly different (P<0.05).

shortest developmental periods of the parasitoid was observed at complete darkness (0L:24D) (for males: F = 156.4.9, df = 2,27, *P* < 0. 05; for females: F = 73.3, df = 2,27, *P* < 0. 05). Where, the egg-larval stage lasted ca.12.9 days for males and 13.9 for females, respectively. While the duration of the prepupal-pupal stage was 8.7 for males and 9.9 days in average for females. The total developmental period lasted 21.6±0.3days for male's vs.23.8±0.02 days for females. When the photoperiod was prolonged, a significant increase in the developmental periods of the immature stages of the parasitoid was observed. At short photoperiod (6L: 18D), the number of days from oviposition to emergence of adults increased to 27.4 and 29.9 days for males and females, respectively. By more prolongation of the photoperiod to 18 L: 6D, the parasitoid required 10 more days for both males and females, compared to those reared under complete darkness. By increasing temperature, the immature developmental time decreased. At complete darkness when the temperature increased to 25ºC, the total developmental period of the immature stages was 18.1 and 19.1 days, for males and female, respectively. At 27ºC, it lasted 17.0 days for males and 18.1 days for females.

The shortest total developmental period of immature stages was observed at 30ºC (16.1 and 17.3 days for males and females, respectively). Thus, the pronounced effect of photoperiod regimes on the immature developmental time observed at 20ºC became non - pronounced at higher temperatures (25, 27 and 30ºC). The total duration of immature developmental period of males was less than that of females irrespective of rearing temperature or photoperiodic regimes. However at 20ºC, the differences were significant at complete darkness (t=6.13, $P < 0.05$) and short photoperiod (t=4.26, $P < 0.05$) and not significance at longer photoperiods. In most cases the differences between the duration of immature developmental period of males and females were not significant at higher temperature 25, 27 and 30ºC (Table 1). Mating of the adult parasitoids occurred shortly after emergence at all the photoperiodic regimes under study period.

DISCUSSION

Detailed knowledge of the insect's life cycle, and how it responds to temperature and photoperiod, is the necessary raw material for mass production of insect parasitoids. It has been determined that the developmental period and adult longevity of parasitoids in particular vary significantly according to temperature (Melton and Browning 1986; Hutchison et al., 1986, Nealis and Fraser, 1988; Hailemichael and Smith, 1994). At 20°C, increasing photoperiod time was correlated with increasing development time of *A. galleriae* while at other temperatures (25, 27, and 30°C) no significant difference was detected. "Although there was no significant effect of temperature, on each photoperiod the development time of *A. galleriae* decreased with increasing temperature. So, the developmental period of the wasp, *A. galleriae* vary significantly according to photoperiod and was temperature dependent. The effect was significantly noticeable at 20°C. The continuous darkness (0L: 24 D) accelerated the development of both the egg-larval or prepupal-pupal stages, while both the short photoperiod (6L: 18D) or continuous light (24L: 0D) slowed down the development. However, the pronounced effect of photoperiod regimes on the immature developmental time observed at 20ºC,changed to non-pronounced effect at higher temperatures (25, 27 and 30ºC).

The influence of photoperiod on the developmental times of the egg-larval and prepupal-pupal stages of the parasite decreased as the temperature increased from 20 to, 25, 27or 30ºC. Where at rearing temperatures other than 20ºC, the difference in the developmental time was not significant. Although, the parasitoid development was suppressed at 20ºC, all formed cocoons of the parasitoids hatched to adults at test rearing conditions. However, in all cases the female pupae issued earlier than males. In preceding investigation on the effect of different photoperiods on the relative speeds of the endo-developmental stages of another non-diapaused wasp, *Microplitis rufiventris* Kok attacking *S.littoralis* larvae, Hegazi and Fuhrer (1985) and Hegazi et al. (1988) reported another trend of results, where the short (6L:18D) photoperiod accelerated the development of wasp larval instars, while continuous darkness (0L:24 D) or longer photperiods (18L:6D or 0L:24D) slowed down the development. The juvenile hormone (JH) has for several years been known to be a necessary factor for maintenance of the larval state (Wigglesworth, 1970; Cymborowski and Stolarz, 1979). Nijhout and Williams (1974) and Takaki and Sakurai (2003) found that JH inhibits secretion of the Prothoracicotropic homone (PTTH). Ciemior et al. (1977), reported that the first periods of PTTH release is regulated by the photoperiod and it seems that the brain is necessary as initiator for maintenance of the developmental rhythms. Vinson and Iwantsch (1980) and Lawrence (1982) reported that during the development of an endoparasitic insect, various metabolic and endocrinological changes occur in the host suggesting that its development is regulated to satisfy the physiological requirements of the parasitoid. Accordingly, the photoperiod at 20ºC induces an indirect effects on the endocrinological balance inside the hosts which may affect the relative speeds of the egg and larval stages of the parasitoid and finally on the emergence time from host individuals. The latter phenomenon may be used in the biological control work program to maximize the optimum mass -rearing conditions which will lead to a refine mass-production of the parasioid. The present results may lead to advancing mass production to the level of economic feasibility which may create new technologies in production of *A. galleriae* wasps that can compete in the open market.

The entire research was conducted based on a practice that beekeepers have of removing honeycombs that are infested with wax moth larvae from beehives and placing them in the open in order to make them more accessible to parasitoids. In the present work, investigating the development of the parasitoid under different photoperiod, suggest that the beekeepers practice might actually be detrimental to parasitism and advice beekeepers to stop placing infested honey combs outside. The hive with bees may be more attractive to parasitoids than an individual honey comb placed away from the hive. Many studies show that parasitoids are more attracted to combinations of specific factors/cues (Donald et al., 1996), thus leaving honey combs outside the hive could mean that some of these cues are not present and possibly results in a reduced parasitism rate. At least in Egypt, wax moth and its parasitoid, *A. galleriae* develop throughout the year in the weak beehives or in infested honey combs in the stores without undergoing diapause. However, Cassier and Cymborowski (1993) state that *G. melonella* overwinters at 18°C, which is probably also true for *A. galleria*. The study showed that, the photoperiods at 20ºC exerted an effect on growth rate of the parasitoid, with some being stimulated (complete darkness) and others being inhibited (short or long photoperiods).The effect of photoperiod on the relative development pattern of the immature stages of well-known non-daipaused insect will be done in our next works.

CONCLUSION

At 20°C, total development time of non- diapausing wasp in Egypt, *A. galleriae*, increased with increasing photophase. This effect was not seen at temperature greater than 20°C. So, the common practice of Egyptian beekeepers to expose wax-moth-infested combs away from the hive to induce parasitoid colonization is not an appropriate management strategy. Also, the study of the interactions between temperature and photoperiod in larval development on diapausing *A. galleriae* wasp in Egypt may offer some insights into the evolution of diapausing *A. galleriae* in Europe.

ACKNOWLEDGEMENT

We are grateful to the editor and anonymous reviewers for their comments and contributions on this manuscript. E. M. Hegazi. thanks the Alexander Von Humboldt Foundation for research donation used in this work.

REFERENCES

Cassier P, Cymborowski B (1993). Physiology of larval diapause in the wax moth, Galleria mellonella: An ultrastructural analysis. Comp. Biochem. Physiol., 105: 679-687.

Ciemior KE, Sehnal F, Cymborowski B (1977). The role of neuro endocrine system as a pacemaker of insect molting cycle. J.Interdiscipl.Cycle Res., 8:301-303.

Cymborowski AW, Stolarz G (1979).The role of juvenile hormone during larval-pupal transformation of *Spodoptera littoralis*: Switchover in the sensitive of the prothracic gland to juvenile hormone .J.insect Physiol., 25: 939-942.

Donald HF, Lucia FG, Justin OS (1996). Specialized parasitoid attracted to a pheromone of ants. Anim. Behaviour, 51: 61-66.

Eizaguirre M, Lopez C , Asin L, Albajes R (1994). Thermoperiodism, photoperiodism and sensitive stage in the diapause induction of *Sesamia nonagrioides* (Lepidoptera:Noctuidae). J.insect Physiol., 40:113-119.

Fantinou AA, Karandinos MG , Tsitsipis JA (1995) . Diapause induction In the *Sesamia nonagrioides* (Lepidoptera: Noctuidae) effect of photoperiod and temperature. Environ. Entomol., 24:1458-1466

Fantinou AA , Tsitsipis JA , Karandinos MG (1996). Effects of short- and long-photoperiods on growth and development of *Sesamia nonagrioides* (Lepidoptera: Noctuidae). Environ. Entomol., 25:1337-1343.

Hailemichael Y, Smith JW (1994). Development and Longevity of *Xanthopimplastemmator* (Hymenoptera: Ichneumonidae) at Constant Temperatures. Ann. Entomol. Soc. Am., 87 (6): 874-878.

Hegazi EM, El-Gayar FH , Rawash I A , Kares EA (1982). Oral and residual toxicities of some insecticides to the adults and full grown larvae of two parasites of the cotton leafworm, *Spodoptera littoralis* (Boisd.). Act. Phytopathologica, 17 (3-4): 301-309.

Hegazi EM, Furhrer E (1985). Instars of *Microplitis rufiventri* (Hym.;Braconidae) and their relative developmental speed under different Photoperiods. Entomophaga., 30 (3): 231-243.

Hegazi EM, Schopf A, Furhrer E (1988).On the developmental synchrony between *Spodoptera littoralis* (Boisd.) and its parasite *Microplitis rufiventris*. J. Insect Physiol., 134: 773-77

Hutchison WD, Butler GD, Martin JM (1986). Temperature-Dependent Development, Mortality, and Longevity of *Microplitis rufiventris* (Hymenoptera: Braconidae), a. Parasitoid of the Beet Armyworm (Lepidoptera: Noctuidae). Ann. Entomol. Soc. Am., 79: 262-265.

Kares EA (1978). Effect of certain insecticides on the cotton leafworm and two parasitoids. M.Sc. Thesis, Fac. of Agric., Alex. Univ., Egypt.

Kulkarni N, Kushwaha D, Mishra V, Paunikar S (2012). Effect of economical modification in artificial diet of great wax moth *Galleria mellonella* (Lepidoptera :Pyralidae), Indian J. Entomol., 74(4): 369-374.

Lawrence 0P (1982) .*Biosteres longicaudatus:* Developmental dependence on host *[Anastrepha suspense}* physiology. Exp.Parasitol., 53: 396-405.

Lopez C, Eizaguirre M, Albajes R (1995). Diapause detection and monitoring in the Mediterranean corn stalk borer. Physiol. Entomol., 20:330-336.

Melton CW, Browning HW (1986). Life History and Reproductive Biology of *Allorhogas pyralophagus* (Hymenoptera: Braconidae), a Parasite Imported for Release Against *Eoreuma loftini* (Lepidoptera: Pyralidae). Ann. Entomol. Soc. Am., 79 (3): 402- 406.

Nealis VG, Fraser S (1988). Rate of Development, Reproduction, and Mass-Rearing of *Apanteles fumiferanae* Vier. (Hymenoptera: Braconidae) Under Controlled Conditions. Can. Ent., 120 (3): 197-204.

Nijhout HF, Williams CM (1974). The control and metamorphosis in *Manduca sexta* (Lepidoptera): Growth of the last instar larvae and the decision to molt.J.Exp.Biol. 61: 481-491.

Roberiro JC (1996). Study of the relationship between *Galleria mellonella* (lepidoptera :Pyralidae) and the parasitoid *Apanteles galleriae* (Hymenoptera :Braconidae). J. Venomous Anim. Toxins, pp.62-62.

Shimamori K (1987).On the biology of *Apanteles galleriae,*a parasite of the two species of wax moths. Honey bee Sci.,8: 107-112.

Singh S (1962) .Beekeeping in India. Indian Council of Agricultural Research, New Delhi, p.214.

Takaki K, Sakurai S (2003). Regulation of prothoracic gland ecdysteroi organic activity leading to pupal metamorphosis. Insect Biochem. Molec. Biol.,33: 1189-1199.

Uckan F, Gulel A (2000) .Effects of host species on some biological characteristics of *Apanteles galleriae* Wilkinson (Hymenoptera: Braconidae). Tr.J.Zoo., 24:105-113.

Uckan F, Gulel A (2001).The effects of cold storage on the adult longevity, fecundity and sex ratio of *Apanteles galleriae* Wilkinson (Hymenoptera: Braconidae). Tr.J.Zoo.,25: 187-191.

Uckan F, Ergin E (2002). Effect of host diet on the immature developmental time, fecundity, sex ratio, adult longevity, and size of *Apanteles galleriae* (Hymenoptera: Braconidae). Environ. Entomol., 31:168-171.

Uckan F, Ergin E (2003). Temperature and food source effects on adult longevity of *Apanteles galleriae* Wilkinson (Hymenoptera:Braconidae). Environ.Entomol., 32: 441-446.

Vinson SB, Iwantsch GF (1980). Host regulation by insect parasitoids. Biol.Rev., 55:143-165.

Watanabe C (1987). Occurrence of *Apanteles galleriae* (Hymenoptera: Braconidae), a parasite of wax moth in Japan. Konty U, 55:165-168.

Whitfield JB, Cameron SA, Ramirez SR, Roesch KS, Messinger S, Taylor OM, Cole D (2001). Review of the *Apanteles* species (Hymenoptera: Braconidae) attacking lepidopterain *Bombus*

(Fervidobombus) (Hymenoptera: Apidae) colonies in the new world, with Description of a new species from South Africa. Ann. Entonmol. Soc.Amr., 94: 851-857.

Wigglesworth VB (1970). Insect Hormones. W.H. Freeman, **San** Francisco,CA USA.p.159.