

*Full Length Research Paper*

# **Persistent of low-level diapause and generation effect in *Microplitis rufiventris* wasps under mass rearing conditions**

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The solitary endoparasitoid, *Microplitis rufiventris* is considered one of the principal wasps on the cotton leaf moth, *Spodoptera littoralis* larvae. It has a strange sort of obligate diapause. The data present an evidence of persistent low-level diapause throughout the year and every year that observed under mass rearing conditions. When continuous rearing was done throughout the year at 25°C and 12/12 h L/D, a small portion of the individuals in each large samples of each generation entered diapause. So, a long-timed experiment that repeated three years and collecting dataset that required regular extensive dissections and microscopic observations of emerged diapausing wasps was carried out. This diapause spontaneously terminates after time periods similar each year, showing an evidence of “time memory” in diapausing wasp behavior. The duration of this time period is taken as a proxy of diapause intensity. This had occurred whether the large number of individuals originated in summer from field-collected host larvae or from three successive years of laboratory-rearing parasitoids. Contrary to non diapausing wasps, diapausing females emerge some days early than males. This is the first report of new sort of obligate diapause in diapause induction and termination in *M. rufiventris* under the same constant rearing conditions. Fecundity of diapaused wasps was diapause intensity dependent. Wasps diapaused for 60-86 days (long diapause) have more number of mature eggs than either of those diapausing for short period (23-57 days) or those of longer diapause (90-165).

**Key words:** *Microplitis rufiventris*, mass rearing conditions, obligate diapause, diapause intensity, generation effect, fecundity.

## **INTRODUCTION**

*Microplitis rufiventris* Kok. (Hymenoptera: Braconidae) is a specialist endoparasitoid of earlier instars of *Spodoptera littoralis* (Boisd.), when they still live in clusters near the

place of egg deposition. However, third instars are preferred (Hegazi et al., 1977). The wasp is usually found with the cotton leafworm, *S. littoralis* larvae and some

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other noctuids in the fields. The parasitoid oviposits a single egg per host and the ontogeny includes three instars which feed on the host's haemolymph. The time of parasitoid development from egg to emergence in *S. littoralis* larvae fed on artificial diet (Hegazi and Führer, 1985) is Ca. 7-8 days when held at  $27\pm 1^\circ\text{C}$  and  $60\pm 5\%$  RH. After reaching the third instar, the larva bores out of its host and spins a cocoon (Khafagi and Hegazi, 2001). The pupal male period is generally 1 day shorter than that for females (7.5 days). Despite the fact that the braconid wasp *M. rufiventris* has been studied extensively, gaps in our knowledge of its biology left behind and need studies. One issue is how the parasitoids diapause, this behavior represents primary part of the life-cycle in many insect species. So, this wasp behavior remains to be discovered that may offer excellent means for the manipulation of wasp populations to man's advantage, e.g. improve quality control of wasp rearing. It is reported that the host (*S. littoralis*) exists in the soil during cold spells without undergoing diapause (Sidibe and Lauge, 1977). Diapause is a period of arrested development during an insect's life cycle. It is an adaptations that parasitoids have evolved to overcome stressful periods and to keep in synchrony with the seasonal occurrence of their life requirements (Tauber et al., 1986). It presents an exceptionally clear important problem in insect biology. Diapause is usually triggered by environmental factors. It can be either obligatory or facultative. In obligate diapause all the individuals in a generation always enter diapause (Wipking, 1990). Obligatory diapause is most often associated with univoltine insects. In facultative diapause some members of a generation enter diapause. Facultative diapause is found in most insects, and is associated with bivoltine or multivoltine insects (Denlinger, 2002; Vladimir, 2006). In fact diapause expression is unknown in a lot of insect species. Knowledge of diapause characteristics in *M. rufiventris* is crucial for developing efficient mass-rearing techniques and fulfilling its potential as a biological control agent to suppress some noctuid pests in the field. The present 3-years study describes for the first time the assessment of diapause development of *M. rufiventris* under mass rearing lab conditions. The present study is not complete. Its main objective is to stimulate further discussion on the important aspect of *M. rufiventris*-diapause behavior and "generation effect" in mass-rearing cultures which in turn might lead to improve quality control in wasp production procedures.

## MATERIALS AND METHODS

### Rearing of *S. littoralis* and *M. rufiventris*

Cultures of *S. littoralis* and the parasitoid *M. rufiventris* were obtained from a laboratory colony established in 2014 at the Department of Entomology, Faculty of Agriculture, Alexandria University. The colonies of *S. littoralis* and *M. rufiventris* are originated from field-collected individuals from crops that

included cotton in Alexandria, Egypt. *S. littoralis* were reared on kidney beans artificial diet (Hegazi et al., 1977). The *M. rufiventris* colony was maintained using 3<sup>rd</sup> instar larvae of *S. littoralis* as hosts, according to methods described by Hegazi and El-Minshawy (1979). Insect rearing and experiment below were conducted in incubators (Hann, Munden, Germany), equipped each with six fluorescent 30-Watt cool white fluorescent tubes. The light onset started at 5 am. The light intensity during photophase was approximately at 1270 foot candles. All cabinets were located in a controlled conditioned room (Hegazi et al., 2017).

During the process of our continuous mass rearing of *M. rufiventris* at  $25\pm 2^\circ\text{C}$ ,  $70\pm 5\%$  RH and photoperiod 12:12 (L:D), we observed that in each large cocoon sample a small portion of the sample did not emerge and passed the normal pupal duration by 1 to 3.5 months and is assumed to be in diapause. The photoperiod 12:12 LD is chosen as in Egypt there is no significant short or long photoperiods and is commonly used for insect rearing.

### Experimental procedure

In 2015, studying was started on this phenomenon where, both of the wasps and hosts were maintained under the same mentioned conditions all the year around to get monthly large samples of well-formed wasp cocoons.

To isolate diapausing wasps from non-diapausing ones, four-six groups of newly molted 3<sup>rd</sup> instar of *S. littoralis* larvae were individually stung weekly by mated middle-aged females of *M. rufiventris*. Each larva was removed immediately after a single oviposition. The parasitized larvae were then transferred into rearing small plastic Petri-dishes (3.8 by 1 cm), containing medium. Larvae that were accidentally parasitized more than once were discarded. The parasitized larvae were checked daily for the well-formed cocoons. Monthly samples of newly formed cocoons were randomly collected and dated rearing month and kept in laboratory at the same conditions for the emergence of parasitoids. This work was continued regularly each month.

To identify the diapausing pupae, each cocoon sample was held for more than two weeks, to allow sufficient time for the non-diapausing wasps to complete their life cycles (7-9 days) and emerge. The emerged adults were counted and removed. When adult emergence was discontinued, the pupae that did not emerge after three weeks, were assumed to be cocoons containing prepupae-pupae in diapause. The numbers of wasps entering diapause and relevant notes were recorded. Each sample of diapausing wasps were counted, dated and allowed to develop under the same conditions. The diapausing wasps were monitored every day for eclosion. Emergence times in cocoon samples collected in different dates were used to calculate diapause intensity (DI). At emergence, they can be sexed by their antennae and by the slightly protruding ovipositor of the females. To maintain genetic diversity, field wasps were periodically added to the colony during the June-August of 2015 and 2016, approximately 100 individuals per week. The experiment was repeated successively in 2016 and 2017.

### Link of diapause intensity to post-diapause reproductive success

The egg load of *M. rufiventris* wasps of three different diapause intensities was studied at emergence and/or one day post-emergence. Samples of diapausing wasps were monitored every day for eclosion. At emergence, only female wasps (20-25 females) that emerged within  $45\pm 3$  (short),  $64\pm 3$  (long) and  $105\pm 3$  days (longer), of each diapause intensity were regularly dissected exactly after emergence and some others one day post-emergence.

*M. rufiventris* female has two ovaries, each with two ovarioles

Mature ovarian eggs were found in the calyx ( $323.3 \pm 6.3 \mu\text{m}$ , eggs to be laid first) and reservoir regions ( $389.1 \pm 20.6 \mu\text{m}$ , ready for transfer to the calyx region by involuntary unidirectional movement of eggs). Oocyte production was determined by dissecting the ovaries of each female and recording the number of mature oocytes (Hegazi et al., 2013) ranged 320-385  $\mu\text{m}$  long. We recorded the egg load by counting the mature number of eggs in the ovarioles at emergence time and at 24h post-emergence. Dissection was made in Ringer's Solution (0.75 g NaCl, 0.035 g KCl, and 0.021 g  $\text{CaCl}_2$  in 100 ml distilled water) under a stereo-zoom microscope (Olympus, model #S111) at a magnification of 60-90X. Using two #0 insect micro-pins, the abdomen of each female was teased apart and ovaries removed. The number of mature eggs presents in the calyx region and the lateral oviducts egg reservoir were counted by teasing the tissues apart so that the individual eggs were released (Hegazi et al., 2013).

### Statistical analysis

Data were subjected to analysis of variance (ANOVA) of one way in completely randomized design for determination of differences between means. Where significant differences occurred, a least significant difference (LSD) test or Student's *t*-test was applied for mean separation. All the analyses were carried out at the 5% significance level.

## RESULTS

### Incidence of diapause under constant mass rearing conditions

The assessments of diapause development of *M. rufiventris* (23-24 generations/ year) under constant laboratory conditions (25°C and 12/12 h L/D) were investigated systematically for 3 successive years during 2015-2017. The intact cocoons remaining after initial ceases in each large laboratory samples /month contain pupae in diapause. Only a small part of the population of each large sample entered diapause. The assumed diapausing wasps were kept under the same mass rearing constant conditions for diapause termination. Among lab monthly samples, Figures 1 and 2 show monthly portions of diapausing *M. rufiventris* pupae and diapause duration in days of diapausing wasps, respectively. The eclosed wasps can be sexed by their antennae and by the slightly protruding ovipositor of the females. The sex ratio of emerged wasps was significantly biased for males. Although the male pupal period of non diapaused wasps is generally 1 day shorter than that of females (7.5 days), in most cases the duration of diapaused female pupae was equal or shorter than males (supplementary data Table 1). In 2015, in a total of 26157 laboratory-reared wasps, 12.9% entered diapause in month 1 (January). The percentage of diapausing wasps in months February, March, April, May, June, July, August, September, October, November and December reached up to 11.3, 8.9, 7.2, 8.3, 5.5, 5.4, 8.3, 6.1, 8.3, 7.8 and 9.8%, respectively (Figure 1). The percentage of diapausing wasps among monthly year

samples was significantly different (ANOVA:  $df = 11, 60$ ;  $F = 46.3$ ;  $P < 0.05$ ). The percentage in January, February, October, November and December was relatively higher and longer in duration compared with those diapausing in middle generations, showing generation effect. Almost similar proportions of diapausing wasps was found among wasps reared in 2016 (ANOVA:  $df = 11, 60$ ;  $F = 576$ ;  $P < 0.05$ ) and even among those of the third year (2017) (ANOVA:  $df = 11, 60$ ;  $F = 116.8$ ;  $P < 0.05$ ) and during the same periods. In first generations of the rearing year and late ones, almost similar proportions of wasps entered in lengthened diapause showing again the time memory in the wasp (Figure 2). So, this behavior under lab rearing condition may directly related to the naturally generation memory in insects during their successive generations, in the study year. The incidence of diapause of *M. rufiventris* in natural outdoor conditions was checked two times. Only 2 of 610 (0.3%) and 3 of 485 (0.6%) of field collected parasitized larvae in August 2015 and July 2016 their pupae entered short diapause under lab condition.

Based on the monthly counts of the diapausing *M. rufiventris* pupae and diapause duration (from the day of pupation to the day of emergence), three overlapping and intersect significant diapause intensities each year were observed under the same constant lab conditions, which may show a kind of timely programmed diapasuse. These categories of diapause intensities (ANOVA:  $df = 2, 27$ ;  $F = 1364.3$ ;  $P < 0.05$ ) depended upon the generation date, that is, time of the year in the lab: Short, long and longer. The short diapause duration ranges from 23 to 57 days, the long ones ranges from 60 to 68 days, while the longer ones develops within 90 to 165 days. However timing of longer diapause was mainly recorded among pupae samples collected during the first and late generations of the rearing year (Figure 2), showing again time memory in the wasps. However, portions of short and long diapause were observed among all monthly samples (Table 1). Some of diapausing females were regularly dissected exactly after emergence and others 1 day-post emergence. Data of female wasps that only emerged within  $45 \pm 3$  days (short diapause),  $64 \pm 3$  days (for long) and  $105 \pm 3$  days (for longer), were selected, taken and presented in Figure 3.

### Egg load of diapausing wasps

Generally, egg loads were significantly influenced by female age. For non diapausing females (control), dissection showed that *M. rufiventris* females at 0 day (aged < 1 h) contained  $62 \pm 3.4$  mature eggs (synovigenic wasp). Females aged 24 h post-emergence had a significantly higher mature egg-load ( $116.2 \pm 4.1$ ) compared with newly emerged ones ( $t = 22.6$ ). Within this age range, the egg load of females diapaused for a short period ( $45 \pm 3$  days) was  $83.2 \pm 2.5$  eggs for newly emerged wasps versus  $96.6 \pm 3.4$  eggs in females aged 24 h post-

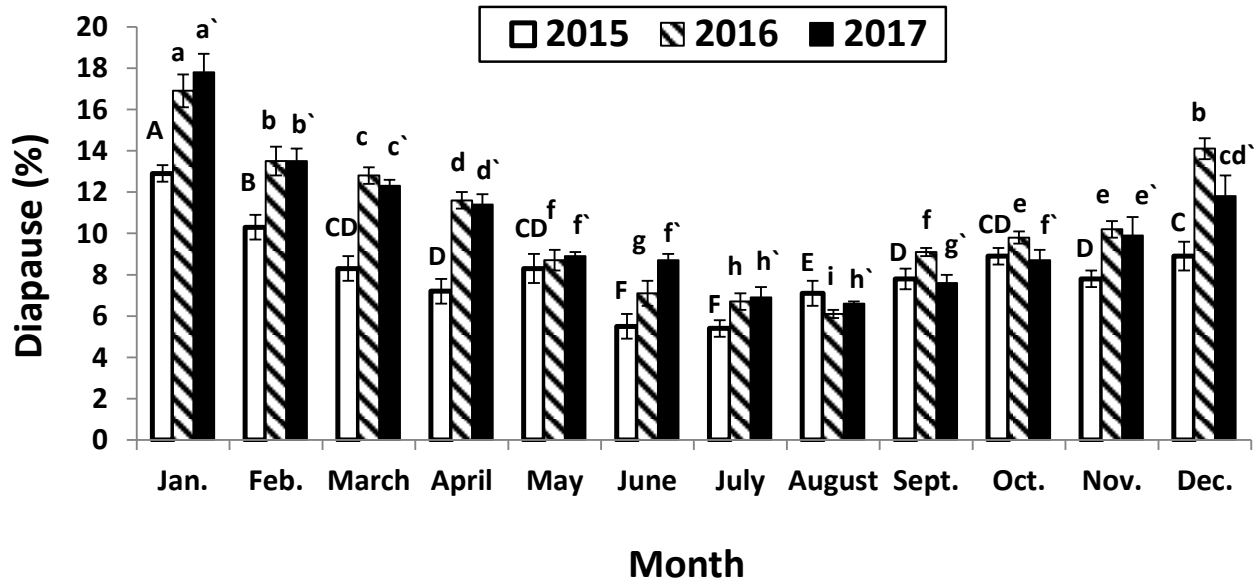


Figure 1. Diapause percentage ( $\pm$  SE) in monthly large lab samples of *M. rufiventris* pupae for wasps produced under constant mass rearing conditions. For each set, bars have the same letters are not significantly different at  $P < 0.05$ .

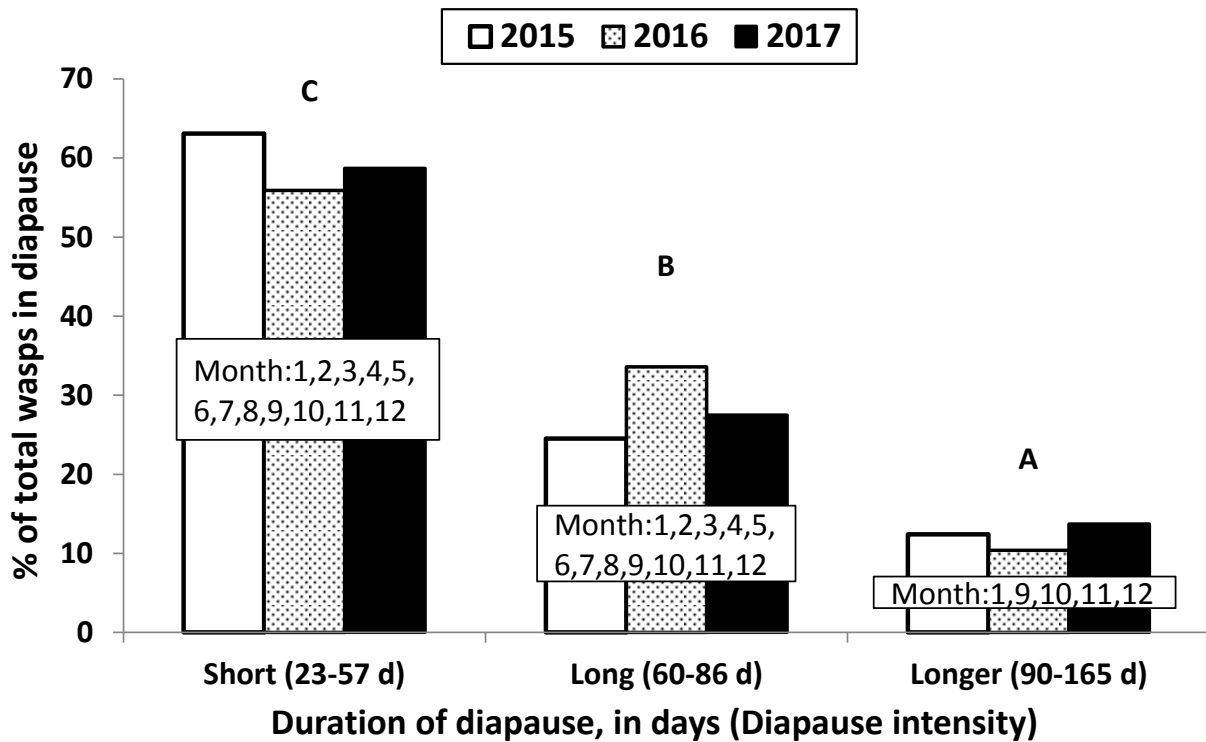


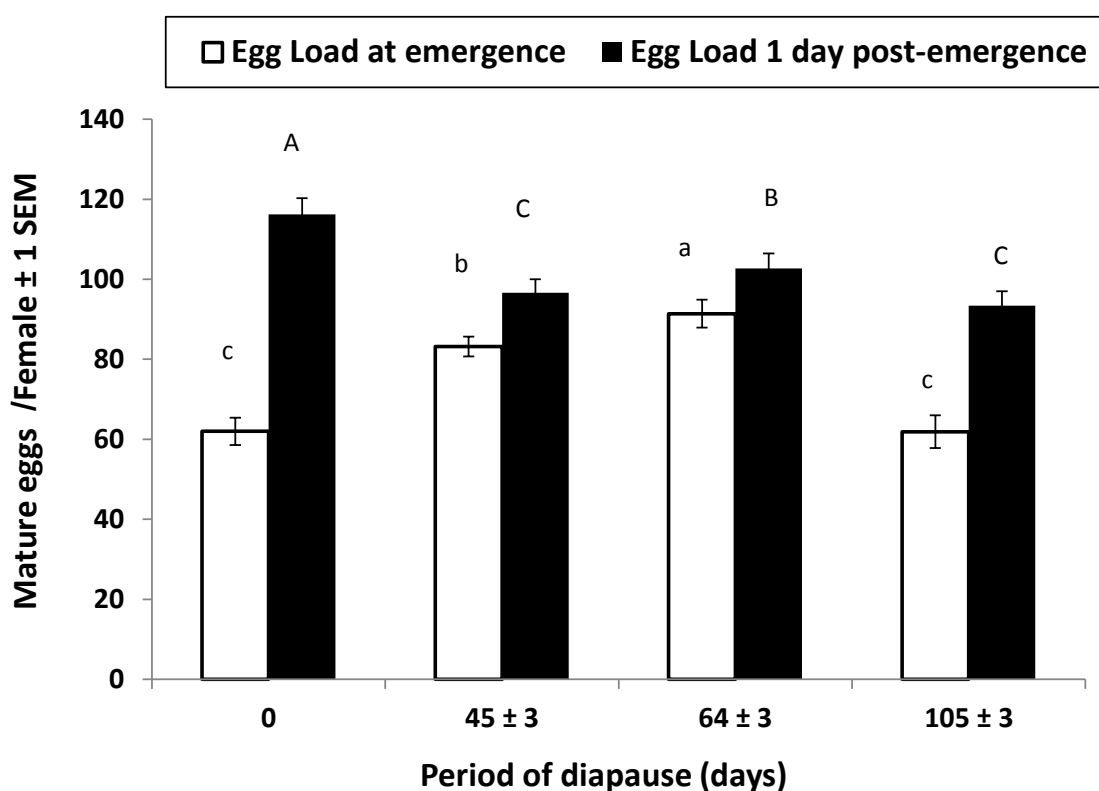
Figure 2. Short (23-57 d), long (60-86 d) and longer (90-165 d) diapause intensities in *M. rufiventris* reared for 3 successive years under constant rearing lab conditions. For diapause mean sets, bars have the same letters are not significantly different at  $P < 0.05$ .

emergence ( $t = 13.4$ ) (Figure 3). The number of mature eggs carried by a female parasitoid diapaused for long period ( $64 \pm 3$  days), was  $91.4 \pm 3.5$  eggs for newly

emerged wasps versus significantly higher egg load of  $107.7 \pm 3.8$  eggs in females aged 24 h post-emergence ( $t = 5.3$ ) (Figure 3). However, for female 24 h post

**Table 1.** Diapausing pupae (%) of *M. rufiventris* in total number of wasp pupae /year and monthly timing of short (23-57 d), long (60-86 d) and longer (90-165 d) diapause among diapaused wasps, under optimal mass rearing conditions.

	2015	2016	2017
Total pupae (No.)	26157	18850	18462
Diapausing wasps %	10.8	11.3	8.1
Diapause intensity:			
A. Short diapause (%)	63.1	55.9	58.7
Time (months)	1,2,3,4,5,6,7,8,9,10,11,12	2,3,4,5,6,8,9,11,12	1,3,4,5,6,7,8,10,11,12
B. Long diapause (%)	24.5	33.8	27.5
Time (months)	1,2,3,4,7,8,10,11,12	1,3,4,6,8,9,10,12	1,2,4,5,9,11,12
C. Longer diapause (%)	12.4	10.3	13.7
Time (months)	1,9,11,12	1,10,11,12	1,11,12



**Figure 3.** Effect of diapause intensity on number of mature eggs/female (mean±SE) in the ovarioles of non-diapaused (0d) and diapaused *M. rufiventris* females of three diapause intensities. For each set, bars have the same letters are not significantly different at P < 0.05.

emergence, mature egg load in *M. rufiventris* females entered diapause for longer period (105±3 days) was 93.4±3.6 eggs versus significantly higher egg load of 116.2±2.5 eggs in non-diapaused or those ones diapaused for long periods (Figure 3). Overall, the differences among number of mature eggs were significant (ANOVA: df =3, 36; F = 30.4; P < 0. 05). The total number of mature eggs carried by a female parasitoid diapaused for long period (64±3 days) was

significantly higher than wasps diapaused for longer period (105±3 days) (ANOVA: df = 3, 36; F = 128.9; P < 0. 05).

The egg load in reservoir region of wasps at eclosion varied significantly (ANOVA: df = 3, 36; F = 12.3; P < 0.01) among wasps of different diapause intensities. (Table 2), which is probably related to the resorption processes in wasps diapaused for longer period but having more mature eggs for wasps diapause for short or

**Table 2.** Mature eggs in the calyx, reservoir regions and total mature eggs in the overioles of non-diapausing (control) and diapausing *M. rufiventris* wasps of three diapause intensities.

Period of diapause (days)	Egg load at emergence			Egg load 1 day post-emergence		
	Calyx eggs	Reservoir eggs	Total	Calyx eggs	Reservoir eggs	Total
0 (control)	0.2±0.1	61.8±3.4	62±3.4 <sup>C</sup>	8.6±1.2	107.6±2.5	116.2±4.1 <sup>a</sup>
Short: 45±3(d)	2.5±0.4	80.7±2.5	83.2±2.5 <sup>B</sup>	6.8±2.2	89.8±3.1	96.6±3.4 <sup>c</sup>
Long: 68±3(d)	2.8±0.3	88.6±3.6	91.4±3.5 <sup>A</sup>	5.9±2.3	96.8±3.9	107.7±3.8 <sup>b</sup>
Longer: 105±(d)	2.6±2.8	61.9±4.1	64.5±3.1 <sup>C</sup>	2.7±0.6	90.7±3.5	93.4±3.6 <sup>c</sup>

For each set, columns bearing the same letters are not significantly different at  $P < 0.05$ .

long period.(Table 2). Wasps diapaused for short or long period contained significantly higher number of mature eggs compared to control wasps. Dissection ovaries of wasps diapaused for longer period contained some collapsed eggs suggesting sign of resorption. Also, the relative distribution of mature eggs in the calyx lumen and oviducts varied among these wasps and was significantly different. The calyx region of diapaused wasps contained at emergence significantly higher numbers of mature eggs (Table 2) than those found in non-diapaused wasps (ANOVA:  $df = 3, 36$ ;  $F = 11.3$ ;  $P < 0.05$ ).

Mortality percentages among non-emerged cocoons ranged from 5.2% in Sept. to 9.3% in January during 2015. The dead cocoons ranged from 4.9% in June to 12.2% in January during 2016 and from 4.9% in August to 9.8% in January of 2017. Of course, some of these dead cocoons (supplementary data Table 1) died during parasitoid development. So it was hard to know whether these wasps died during diapause period or before.

## DISCUSSION

Photoperiod and temperature are the most important factors initiating and regulating diapause in polyvoltine insects, including noctuids and parasitoids (Tauber et al., 1983; Ishii et al., 2000). Information on regulation of diapause in *M. rufiventris* has an important bearing and high practical value on the use of this parasitoid for mass rearing and biological control of some noctuid insects. There is no clear understanding of the mechanism of diapause regulation in *M. rufiventris*. So, to gain knowledge of its diapause behavior, the incidence of diapause in *M. rufiventris* wasps was studied by monthly large-scale lab samplings over a period of 3 successive years under constant mass rearing conditions (25°C and 12/12 h L/D). Under these conditions, significant Persistent low-level diapause was observed throughout the year and every year. On the contrary, continuous rearing of both host (*Hadena bicruris*) and its Parasitoid (*M. tristis*) was undertaken at 25°C and 14/10 h L/D. But both host and parasitoid did not enter diapause (Elzinga et al., 2002). In case of *M. mediator* (Wenxiang et al.,

2008), no diapause was observed at temperatures above 20°C regardless of the photoperiod. Only a small part of the population entered diapause at 20°C under short day lengths of light: dark 8:16-12:12.

The period required for the completion of diapause development was used as an index of the intensity (Hodek, 1983; Tauber et al., 1986). Three categories of diapause intensities (DIs) were detected in *M. rufiventris* pupae: Short, long and longer “prolonged”. It is reported that, the time of diapause is mostly dependent on temperature, photoperiodic, moisture and food availability. The three year of *M. rufiventris* diapaused pupae were obtained under constant laboratory rearing conditions (25±2°C, 70±5% RH and photoperiod 12:12 (L:D) and diet),so the present study may provide an evidence that the duration of *M. rufiventris* diapause significantly depends on the generation time “time memory” during the laboratory rearing year. Evidence of time memory comes from the fact that the duration of diapause was significantly longer among wasps obtained in the early and late generations each year while the shortened ones were mostly in the middle time of the year” middle generations” at the same conditions of 25°C and 12/12 h L/D. Showing that generation time “time memory” affects the duration of diapause. Short and long diapauses were detected among wasp samples of the rearing year. This response was repeated during the three successive years, suggesting generation effect. The physiological mechanisms of longer diapause are not clear. Many insects require cool temperature for successful completion of diapause development (Hodek, 2002). However, studies of Hodek (2002); Damos and Savopoulou-Soultani (2010) and Mironidis and Savopoulou-Soultani (2012) show that chilling is not a prerequisite for the completion of hibernation “winter” diapause and diapause completion progresses well at intermediate or high temperatures, sometimes it is even stimulated by high or increasing temperatures. On other hand, temperature, photoperiod and moisture have been indicated as important factors for termination of the diapause (Alvi and Momoi, 1994; Ishii et al., 2000; Seymour and Jones, 2000) but this appears to be very species-specific.

On the contrary, in the present case of *M. rufiventris*,

diapause completion progresses spontaneously at the same conditions that induced diapause, and at conditions favorable to continuous development for parasitoid mass rearing among other wasps. Also, the DI could vary among individuals of parasitoid wasps depending on the generation time in each rearing year “generation effect”. The study may provide an evidence that the Egyptian population of the *M. rufiventris* have a genetically determined new kind of obligate diapause with three different intensities in almost within similar periods of the year.

Tauber et al. (1986) and Danks (1987) reported that diapause may be defined as a genetically programmed, neurohormonally mediated, dynamic state of low metabolic activity during which morphogenesis ceases or significantly slows down. *M. rufiventris* is synovigenic parasitoid that do not have a full complement of egg load at eclosion and that continue to mature eggs throughout adult life (Hegazi et al., 2013). The egg load in reservoir region varied significantly among wasps of different DIs which was probably related to the resorption processes among old and or new produced mature eggs in wasp’s diapausing for longer period and producing new mature eggs for wasp’s diapause for short or long period. It seems that the female adjusts the number of her matured eggs in response to changing in diapause intensity.

The results may indicate that there were two types of wasp prepuae-pupae differed physiologically, ones are non-diapausing developed normally and ones have different memory mechanisms to deal with mature eggs according to type of ID. Anyhow, diapause in *M. rufiventris* did not arrest its egg production (Tauber et al., 1986). The causes of diapause in parasitic Hymenoptera are not simple. In many species the individuals may enter a state of diapause at a time when the environment is favorable to continuous development and increase of the species (Flanders, 1944; Simmonds, 1948).

Diapause, a state of arrested development, was one of the major adaptations that parasitoids have evolved to overcome stressful periods and to keep in synchrony with the seasonal occurrence of their biotic requirements (Tauber et al., 1986). In biological control programs, however, diapause can be a major obstacle preventing the continuous rearing of hosts and their parasitoids or predators, and also by lowering the efficacy of the natural enemies released (Salom et al., 2001; Velarde et al., 2002; Musolin et al., 2004). On the other hand, diapause has been recognized as an asset because it can allow or enhance the storage of biocontrol agents (Rundle et al., 2004; Mehrnejad and Copland, 2005; Broufas et al., 2006). Danks (1987) added that diapause is one of the basic means by which insects cope with unfavorable environmental conditions. Diapause plays role in insect life cycles by allowing survival during adverse seasonal conditions as well as synchronizing life cycles with the period of mate and food availability (Posledovich et al., and small part of wasps that entered Persistent low-level diapause did not affect the mass rearing program.

Variation in the intensity of diapause among individuals of *Microplitis* can be attributed to the “timely programmed” of genetic component of individual life cycle traits and environment (Beckage, 1985; Wipking, 1990).

The host, *S. littoralis* develops throughout the year, overwintering in the soil during cold spells and does not undergo diapause (Sidibe and Lauge, 1977; Salem and Salama, 1985). The internal larval parasitoid *M. rufiventris* usually found with the leafworm in the fields. So, this adaptations in diapause of three intensities that parasitoid wasps have, evolved in order to synchronize their life cycle with that of their hosts all the year around. By doing so, it would be possible to have the necessary number of adults ready at the precise time of the year. Understanding the interaction between *S. littoralis* and its parasitoid may improve the rearing capability and allow more accurate timing of parasitoid releases.

Diapause is a dynamic process consisting of several successive phases. The conception and naming of the phases (diapause induction, preparation, initiation, maintenance, termination and post-diapause quiescence) is unsettled and, sometimes, ambiguous in the literature. The specific progression through diapause phases in each species, population (genotype), or even individual, is based on (thus far largely unknown) physiological processes (Vladimir, 2006). The study on *M. rufiventris* wasp presents the first ambiguous evidence that diapause in the wasp could be observed under optimal mass rearing conditions without clear successive diapause phases. Where small portion of the individuals in each large sample of each generation developed diapause and spontaneously terminate diapause under the same rearing conditions and showing three diapause intensities, each “timely programmed”. The study may provide an evidence that the wasp has a genetically timed and determined diapause in a portion of each of its several annual generations.

## Conclusions

The study of *M. rufiventris* presents the first ambiguous evidence that in the wasp, a persistent low-level diapause could be observed throughout the year and every year under constant laboratory mass rearing conditions. The egg load varied significantly among wasps of different diapause intensities which was low in wasps diapausing for longer period, but higher among wasps diapaused for short or long periods.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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**Supplementary Table 1.** Portions of diapausing (mean±SD) and diapause duration of *M. rufiventris* in large samples/month of well-formed cocoons after rearing under optimum mass conditions in 2015, 2016 and 2017.

Month	Sample (No.)	Non-emerged wasps (%) (mean±SD)		Diapause duration (d)			
		Dead	Diapaused	Male		Female	
				Range	Mean±SD	Range	Mean±SD
Jan-15	2226	9.3±0.7	12.9±0.4	33-99	57.9±2.6	39-98	56.6±3.8
2016	1659	12.2±0.6	16.9±0.8	31-96	53.1±2.3	30-101	52.7±3.7
2017	1228	9.8±1.1	17.8±0.9	38-85	54.2±2.4	43-70	53.3±2.6
Feb-15	1213	8.6±0.6	10.3±0.6	35-84	49.1±1.8	35-69	48.1±2.0
2016	1122	7.7±0.5	13.5±0.7	38-81	49.05±1.7	31-60	47.9±1.8
2017	1096	7.2±1.2	13.5±0.6	33-76	53.0±2.5	38-71	49.0±2.0
Mar-15	957	6.1±0.4	8.3±0.6	30-67	47.0±2.3	30-68	44.6±3.4
2016	1743	5.2±0.6	12.8±0.4	33-81	45.9±2.1	30-78	44.05±2.4
2017	1172	9.1±0.4	12.3±0.3	28-50	40.0±1.1	32-48	37.3±1.2
Apr-15	1998	6.3±0.4	7.2±0.6	40-72	43.5±1.5	35-46	40.4±1.4
2016	1192	10.4±0.3	11.6±0.4	27-66	45.6±2.2	27-66	45.6±2.9
2017	981	7.7±0.6	11.4±0.5	27-50	35.3±1.4	27-46	34.9±1.3
May-15	2051	7.3±0.6	8.3±0.7	30-71	38.5±3.1	30-66	37.5±2.9
2016	2198	6.3±0.4	8.7±0.5	23-49	36.8±1.1	21-50	36.2±0.7
2017	959	5.3±0.5	8.9±0.2	24-50	36.2±1.7	25-60	37.8±1.5
Jun-15	1694	8.7±0.6	5.5±0.6	28-70	42.5±2.5	30-68	40.2±2.4
2016	1823	5.7±0.5	7.1±0.6	23-47	58.8±0.4	25-60	29.7±0.7
2017	1393	6.7±0.6	8.7±0.3	27-64	41.7±2.9	27-67	39.6±2.3
Jul-15	1228	9.4±0.2	5.4±0.4	30-60	45.3±3.8	30-60	42.0±3.2
2016	1427	4.9±0.4	6.7±0.4	30-82	42.9±2.6	28-63	41.4±3.2
2017	1364	8.1±0.6	6.9±0.5	30-69	46.2±2.7	35-66	43.7±1.7
August 2015	858	7.6±0.6	7.1±0.6	35-72	51.0±3.9	40-73	45.8±3.8
2016	1556	5.5±0.5	6.1±0.2	33-71	46.7±2.8	34-71	45.6±3.5
2017	1129	4.9±0.7	6.6±0.1	35-76	51.3±4.2	36-74	48.1±3.5
Sept. 2015	1705	5.2±0.6	7.8±0.5	40-100	66.6±5.3	35-106	58.1±3.6
2016	1042	8.8±0.6	9.1±0.2	33-71	50.8±2.6	35-76	50.3±2.5
2017	1493	7.3±0.8	7.6±0.4	34-100	61.9±4.9	38-100	61.5±3.9
Oct. 2015	3153	6.6±0.4	8.9±0.4	40-100	72.8±5.6	50-102	59.6±3.1
2016	2106	7.5±0.7	9.8±0.3	35-101	68.2±4.6	49-105	60.7±3.5
2017	2453	6.7±0.7	8.7±0.5	39-101	65.7±4.4	42-100	62.4±4.1
Nov. 2015	4512	8.1±0.6	7.8±0.4	30-129	68.2±5.5	35-104	61.6±6.1
2016	2137	7.9±0.8	10.2±0.4	33-71	67.7±5.6	33-103	59.7±4.7
2017	2889	3.9±0.9	9.9±0.9	40-100	71.9±5.5	37-110	64.1±4.1
Dec. 2015	4562	6.5±0.2	8.9±0.7	30-165	75.9±5.2	25-121	69.8±6.4
2016	845	7.7±0.9	14.1±0.5	32-69	78.3±4.9	30-120	68.0±6.1
2017	2305	8.1±0.4	11.8±1.0	35-154	79.6±5.8	42-125	71.8±4.3