

RESEARCH

Biological Characteristics of *Grapholita molesta* (Lepidoptera: Tortricidae) Induced to Diapause in Laboratory

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ABSTRACT. In southern Brazil, *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae) uses diapause as survival strategy during the winter (May–August). In our study, we evaluated the effect of diapause on biological characteristics of the species for 4 months in laboratory. Newly hatched larvae of *G. molesta* were induced to diapause changing the photoperiod and temperature (T) ($12 \pm 1^\circ\text{C}$), relative humidity (RH) ($70 \pm 10\%$), and a photophase of 12 h and, when they started diapause in the prepupal stage, the conditions were kept for 4 months. Afterwards, the insects were induced to finalize the diapause process at $T 25 \pm 1^\circ\text{C}$, RH $70 \pm 10\%$, and a photophase of 16 h. We evaluated the duration and viability of the larval stages and pupae, pupae weight at 24 h and sex ratio (sr), periods of preoviposition, oviposition, and postoviposition; adult life span (males and females); fecundity (daily and total); embryonic period duration and eggs viability, comparing the data with insects nondiapause. The results show that diapause greatly affected the viability of pupal–adult stages of the population (21.8%) when compared with insects' nondiapause (80.0%). Total fecundity (83.0 eggs) and mean life span (12.0 d) of insects diapause was significantly lower compared with insects nondiapause (173.0 and 17.0), respectively. However, these differences were not observed in the sr, which was similar to insects diapause (sr = 0.41) and insects nondiapause (sr = 0.49). The diapause induced for 4 months negatively affects reproduction and life span of adults of *G. molesta*.

Key Words: Oriental fruit, metabolic expenditure, reproduction rate, photophase, rosaceae

Fruit trees from temperate climate, especially apple and peach trees, have a dormant period (numbness) when their growth is limited. The plants use this phase to accumulate reserves in the form of carbohydrates to be used during the next growing season (Camelatto 1990). A process of deep dormancy also occurs in insects, called "diapause" and is manifested according to the season, based on the perception of signals in the long quiescent period. Diapause corresponds to a temporary state of reduced metabolism that occurs in response to sudden and particular environmental conditions (Kostal 2006). Out of the diapause period, the dormant insect resumes its growth and development right after the end of the unfavorable period (Kostal 2006).

In the case of *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae), diapause is induced mainly by the short photoperiod (12 h) in combination with low temperatures, occurring during the fifth instar (Dickson 1949). Diapause helps the insects to synchronize its biological characteristics with the available food source and reproduction, allowing the survival of *G. molesta* in fruit orchards during winter (Arioli et al. 2005, Poltronieri et al. 2008).

Although diapause is considered beneficial for the insects because it allow its survival during harsh periods, it is a strategy with high metabolic expenditure, affecting negatively the reproductive potential in the prediapause period (Hahan and Delinger 2007). This occurs in some species of Lepidoptera (Gebre-Amlak 1989), Coleoptera (Ishihara and Shimada 1995), Acari (Kroon and Veenendaal 1998), and Hymenoptera (Eilers and Van Alphen 2002), and the studies show that after diapause, fecundity was reduced.

Studies on effects of diapause on *G. molesta* focus on factors that control the induction and maintenance of the process (Dickson 1949); however, the effect of this strategy on the biotic potential of the insects after this stage is still unknown. *G. molesta* has several generations

during the year (Grellmann et al. 1992, Hickel et al. 2003, Arioli et al. 2005); therefore, if there is a reduction of the biotic potential of the postdiapause generation, associated or not to the different effect on the mortality between the insects sexes, adjustments of procedures for pest control of this generation are required, rationalizing the use of insecticides (Arioli et al. 2007). This study evaluated the effect of diapause of *G. molesta* on insects' mortality and species reproduction in laboratory.

Materials and Methods

The experiments were conducted at the Laboratory of Insects Biology and Biological Control (LBICB) from the Department of Phytosanitary, at the College of Agriculture "Eliseu Maciel", Universidade Federal de Pelotas, in the municipality of Capão do Leão, Rio Grande do Sul State, Brazil.

Newly hatched larvae of *G. molesta* (<24 h), reared at LBICB according to the methodology of Arioli et al. (2007), were transferred individually to glass tubes (2.5 cm in diameter \times 8.5 cm in height), fed with artificial diet of corn meal and the tubes were later closed with cotton. After the inoculation of the larvae, 150 tubes were kept in an acclimatized chamber ($25 \pm 1^\circ\text{C}$, relative humidity [RH] $70 \pm 10\%$ and a photophase of 16 h) and the insects were considered nondiapause. Meanwhile, 720 glass tubes (2.5 cm in diameter \times 8.5 cm in height) containing the artificial diet described by Arioli et al. (2007) were inoculated with newly hatched larvae (<24 h) and kept at $12 \pm 1^\circ\text{C}$, RH $70 \pm 10\%$, and a photophase of 12 h. We used a greater number of larvae for insects that were induced to diapause because of the high mortality rate caused by the low temperature (12°C) (Grellmann et al. 1992).

When the insect nondiapause reached the prepupal stage, they were kept under the same conditions of the larval stage ($T 25 \pm 1^\circ\text{C}$, RH

70 ± 10%, and a photophase of 16 h). The viability and period duration were registered. The insect diapause remained in the fifth instar (Dickson 1949, Russel and Bouzoane 1989) for 4 months, simulating the off-season period for the apple and peach crops in southern Brazil. In the period when the larvae remained in diapause, the artificial diet was changed whenever necessary, keeping it as a humidity source, once the insect was inside its cocoon and did not feed. The transition from the diapause stage was made by changing the temperature gradually to avoid possible thermal shock. First, was changed the temperature to 18 ± 1°C and a photophase of 14 h per day and on the following day to T 25 ± 1°C, RH 70 ± 10%, and a photophase of 16 h. Afterwards, the larvae resumed their growth changing from the pupal stage to adult emergence.

The duration and viability of larval stages and pupae, pupa weight with 24 h, and sr were evaluated. After adult emergence, we assessed the periods of preoviposition, oviposition, and postoviposition; adult life span (males and females); fecundity (daily and total); duration of embryonic period; and egg viability. To evaluate fecundity and adult life span, 25 insect pairs at the same age were placed in PVC cages (15 cm height × 15 cm in diameter) in each treatment, and were registered daily adult mortality and number of eggs laid. To determine egg viability and duration of the embryonic period, 200 eggs per treatment with 10 repetitions of 20 eggs were used.

Statistical Analyses. The data were tested for normality using the Shapiro-Wilk (1965) test and for homoscedasticity using the Hartley (1950) and Bartlett (1937) tests. Data on stage duration, pupa weight, life span, and fecundity that did not show normal distribution or homogeneity of variance were transformed to $x + 0.5$ root and the data expressed in percentage (viabilities) were converted to $\arcsin x/100^{0.5}$. The means of the treatments were compared in the *t*-test ($P < 0.05$), using software using SAS (SAS Institute 2000) and the sex ratio (sr) was calculated in Qui-quadratic test (X^2) ($P \leq 0.05$).

Results

The photoperiod used induced diapause in 100% of the larvae that reached the prepupae stage.

Insects that entered diapause remained for 115 d in the prepupal stage at a temperature of 12°C and a photoperiod of 12 h. The egg viability from insect nondiapause (81.7%) was higher compared with those from insect diapause (60%) (Fig. 1). The viability of the larval stage in insect nondiapause was significantly higher (85%) when compared with insect diapause (37.8%) (Fig. 1). The viability of the larval–adult period was 21.8% for insect diapause, significantly lower when compared with insect nondiapause (80.0%) (Fig. 1). Furthermore, we

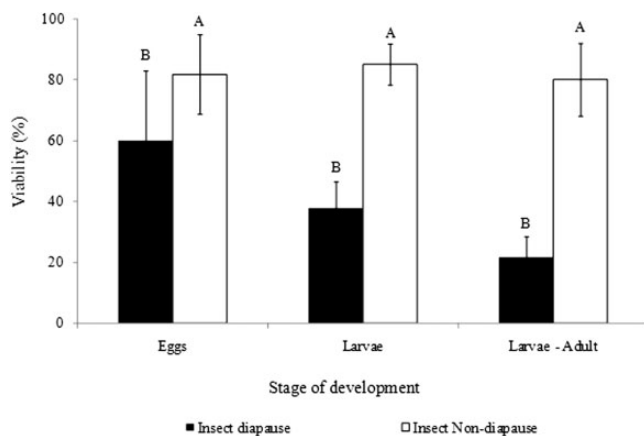


Fig. 1. Average viability (\pm SE) of egg, larvae, and larvae – of insect diapause and insect nondiapause of *G. molesta* in laboratory. There was statistical difference (*t*-test at $P \leq 0.05$) between insect diapause and insect nondiapause.

found that 17.3% of the larvae that underwent diapause showed symptoms associated with “larvae tissues deterioration—LTD” (Figs. 2C–F). Larvae that expressed the symptom had no discoloration of the body (tegument), compared with other diapaused larvae at the same age. This was the only significant mortality factor of prepupae diapause. Hypothetically, the longer the period, the greater the LTD incidence, reducing the insects’ survival. These results support the hypothesis that photoperiodic time measurement in the *G. molesta* is based on a qualitative principle. However, the measurement of photoperiod in *G. molesta* will be performed in future experiments.

The sr obtained between insect diapause (sr = 0.41) and nondiapause (sr = 0.49) was not significant ($X^2 = 1.57$, $P \leq 0.05$), indicating that the insects survival during this period was similar for both sexes. A sr of 0.5 indicates a 1:1 ratio (male:female), which means that each male captured in pheromone traps corresponds to a free female in the field. The pupae weight was also affected by diapause (Table 1). Insect diapause generated male pupae with a mean weight of 11.6 mg and females with 12.4 (Table 1). When compared with insect nondiapause (14.6 and 18.0 mg) for male and female pupae (Table 1), meaning a weight reduction of 21.4% for male and 33.3% in female pupae.

Diapause did not change the preoviposition (4 d), postoviposition (3.1 d), and embryonic (4.1 d) periods (Fig. 3). However, insects’ nondiapause shows an oviposition period approximately twice as long when compared with insects’ diapause (Fig. 3). The fact that diapause does not modify the embryonic period indicates that the offspring is probably not affected by the physiological condition of the parents. Diapause affected reproduction of *G. molesta*, and insects’ nondiapause shows the double of fertility compared with insects diapause (Table 1). This effect can be associated with the low survival of females’ diapause 12.6 d, significantly different from females’ nondiapause with an average life span of 18.2 d (Fig. 3).

Discussion

The influence of temperature and photoperiod on the development of *G. molesta* is well explained by Dickson (1949). According to the author, although the temperature increases the sensitivity of larvae, it does not induce to diapause. Photoperiod is the factor that induces to diapause. Photoperiod and temperature used in this study to induce to diapause in *G. molesta* were chosen based on literature information. The photoperiod (12 h) was based on the studies conducted by Dickson (1949) and temperature (12°C) was based on regular climate conditions occurring in the municipalities of São Joaquim (Santa Catarina State, Brazil) and Vacaria (Rio Grande do Sul State, Brazil) (CPACT 2008), during the months when the insect enters diapause (Arioli et al. 2004, Poltronieri et al. 2008). These municipalities are important producing regions of temperate fruits in southern Brazil.

The photoperiod used induced diapause in 100% of the larvae, in accordance with the findings of Dickson (1949). The author states that even inside the fruit, the insect perceives changes in day length, inducing the larva to enter diapause. The insect is capable of surviving this period without feeding due to the reduced metabolic activity and the use of food reserves (Hahan and Delinger 2007). Furthermore, the immobility of larvae helps to save energy. This effect was also observed with *Lobesia botrana* Den. Y Schiff. (Lepidoptera: Tortricidae) where the diapause of larvae did not affect the transfer of spermatophores or egg viability in adult insects (Torres-Vila et al. 1996). A reduced larval viability (18%) was also recorded by Grellmann et al. (1992), keeping the insect at 20°C. This high larval mortality observed in insect diapause was attributed to the low temperature used in the experiment that is close to base temperature 9.04 of the species (Grellmann et al. 1992).

The lower viability of the larval–adult period of insect diapause, when compared with insect nondiapause were similar with the results found by Sausen et al. (2011), who observed a reduction in larval viability when the insects were exposed to a photoperiod of 12 h. This highlights the metabolic expenditure of insects during the diapause period, once it changes metabolic pathways and physiological processes in the

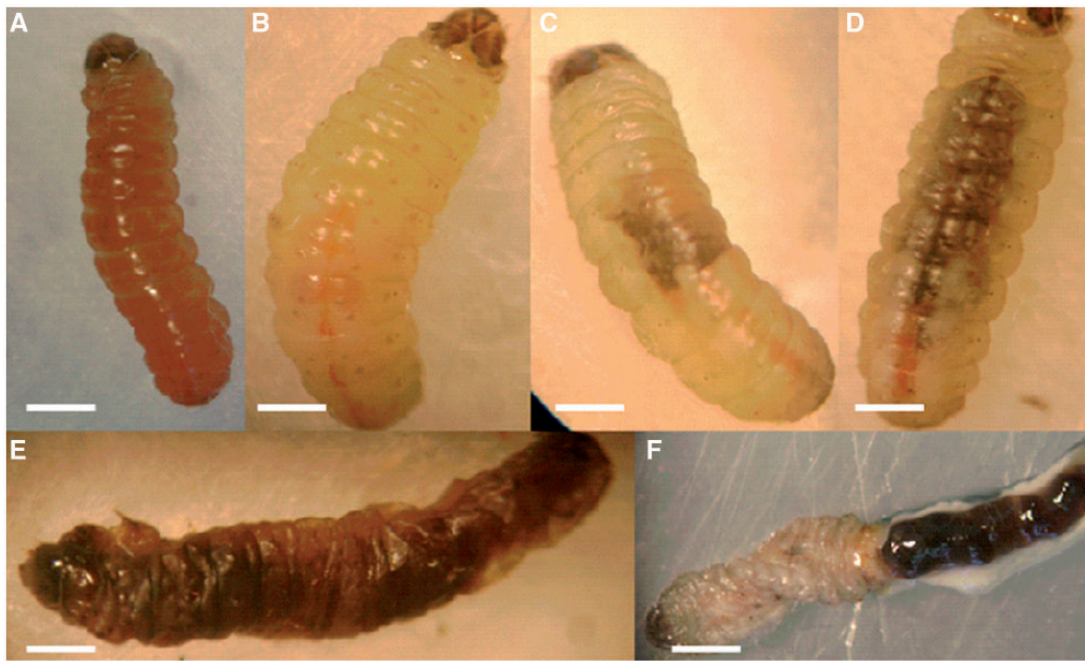


Fig. 2. Symptoms of LTD in *G. molesta*. (A) prepupae of insect nondiapause (T 25 ± 1°C, RH 70 ± 10%, and photophase 16 h). Bar size = 1.87 mm; (B) normal prepupa after 4 months of diapause (12 ± 1°C, RH 70 ± 10%, and photophase 126 h). Bar size = 1.3687 mm; (C) live prepupa with initial and advanced symptom (D) of LTD. Bar size = 1.25 mm; (E and F) dead prepupa with LTD symptom. Bar size E = 1.87 mm. Bar size F = 2.41 mm.

Table 1. Pupal weight (males and females) and daily and total fecundity of *G. molesta* diapausing and nondiapausing in laborator

Biological parameter	Diapausing ^a	Nondiapausing ^a
Pupal weight (males)	11.6 ± 3.00 A	14.6 ± 2.00 A
Pupal weight (females)	12.4 ± 2.00 B	18.0 ± 3.00 A
Daily fecundity	14.2 ± 3.30 A	13.7 ± 7.30 A
Total fecundity	83.0 ± 60.5 B	173.0 ± 65.1 A

^aValues represent means ± SE. There was statistical difference (*t*-test, $P \leq 0.05$) between insect diapause and insect nondiapause.

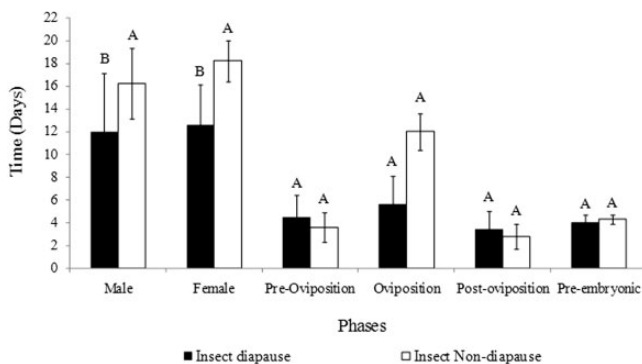


Fig. 3. Average (±SE) life span of males and females, preoviposition, oviposition, postoviposition and pre-embryonic (days) of insect diapause and insect nondiapause of *G. molesta* in laboratory. There was statistical difference (*t*-test at $P \leq 0.05$) between insect diapause and insect nondiapause.

first generation postdiapause (Kostal 2006), causing that occur symptoms associated with LTD. Larvae that expressed the symptom had no discoloration of the body (tegument), compared with other diapaused larvae at the same age. This was the only significant mortality factor of prepupae diapause. Hypothetically, the longer the period, the greater

LTD incidence, reducing the insects' survival. However, this kind of symptom was not observed in larvae in the field.

The sr obtained between insect diapause and nondiapause was not significant. The srs found in this study are similar to those obtained by other authors that reared the insect in quince fruits ($sr = 0.45$) and in artificial corn flour diet ($sr = 0.42$) (Rosenthal et al. 1994, Arioli et al. 2007). A difference in sr is due to diapause results in practical implications that should be considered in management strategies of the insect in temperate fruits, mainly regarding the adequacy of control for first generation postdiapause. The hypothesis that diapause negatively affects the survival of females of *G. molesta* and that in the first generation (postdiapause), greater number of males in the population (Aranda 1942) was rejected in this study.

The pupae weight was also affected by diapause. This confirms the energy expenditure of larvae to maintain the process, corroborating studies of Torres-Vila et al. (1996), who reported an energy expenditure of larvae of *L. botrana* when subjected to diapause. However, the diapause did not change the preoviposition, postoviposition, and embryonic periods, only oviposition period. The fact that diapause does not modify the embryonic period indicates that the offspring is probably not affected by the physiological condition of the parents in the first generation postdiapause. Similar results were also observed in females of *G. molesta* collected from larvae diapause from a peach orchard in Uruguay, where an average of 27 eggs were laid when compared with females of the fourth generation of insects nondiapause (53 eggs) (Aranda 1942). The reduction in fertility was also observed for *Kytorhinus sharpianus* (Coleoptera: Bruchidae) (Ishihara and Shimada, 1995), *Tetranychus urticae* (Acari: Tetranychidae) (Kroon and Veenendaal, 1998), and *Asobara tabida* (Hymenoptera: Braconidae) (Ellers and Van Alphen 2002). In general, the longer the duration of diapause, the higher the energy expenditure, resulting in reduced life span and fecundity of adult (Torres-Vila et al. 1996, Ellers and Van Alphen 2002).

The reduction of the oviposition in the first generation postdiapause can be attributed to the use of metabolic reserves stored for larval survival during diapause (Kroon and Veenendaal 1998, Ellers and

Van Alphen 2002). Due to the reduction in female life span and fecundity after diapause induction, can be infer that the population of the first flight (after diapause) would be more susceptible to behavioral control (sex confusion) than subsequent generations would. This is because the generation after diapause has shorter life span, which decreases the chances of mating success. Even if they do mate, females have lower biotic potential and therefore do not damage the peach and apple cultures (Arioli et al. 2005, Arioli et al. 2007). According to these authors, the population peak postdiapause cannot synchronize with available food sources (pointers or fruits). However, in this period, larvae of oriental fruit moth are found feeding on “burrknots” (aerial root), assuming that they are a source of insect food at this stage, which would allow the development of subsequent generations in the orchard (Arioli et al. 2007, Santos and Leolato 2011, Bisognin et al. 2012). The responses observed in this study indicate that diapause has an energy expenditure for *G. molesta* reducing fertility and life span. However, despite the reduction in the reproductive capacity of the insects, diapause can be a viable strategy for laboratories aiming to store insects for at least 4 months with reduced labor force.

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