

Research Article

Storage of the egg-larval parasitoid, *Ascogaster quadridentata* (Hym.: Braconidae) inside its host larvae, *Cydia pomonella* under diapause conditions

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Abstract: The egg-larval parasitoid, *Ascogaster quadridentata* Wesmael (Hym.: Braconidae) was stored for six months in the mature larvae of codling moth (CM), *Cydia pomonella* (L.) (Lep.: Tortricidae), under diapause conditions. Percentage of parasitoid adults (wasps) emerged from CM larvae reared under diapause conditions was about 86.6%. The biological characteristics (weight and longevity) were similar for both wasps that were treated under standard and diapause conditions. The data showed that percentage of parasitism for females emerged from standard conditions was 86.4%, while increased significantly to 97.8% for females emerged from diapause conditions. These findings may contribute to the mass rearing of *A. quadridentata*.

Keywords: codling moth, biological control, storing the parasitoid

Introduction

In the past decades, many novel strategies, such as mating disruption (Mansour and Mohamad, 2001; Judd and Gardiner, 2005; Aghdam, 2015; Isci, 2016), the sterile insect technique (Bloem *et al.*, 2006; Horner *et al.*, 2016), microbial control (Odendaal *et al.*, 2015) and integrated pest management (Damos *et al.*, 2015) have been developed to control *Cydia* species. The success of these methods depends on population density (Carde and Minks, 1995; Mansour and Mohamad, 2001; Plucienik, 2013). These strategies are not only environmentally friendly methods, but they are also harmless to beneficial insects (Kabir and Rainis, 2015). *Ascogaster quadridentata* Wesmael (Hym.: Braconidae) is an egg-larval parasitoid of the codling moth (CM) *Cydia pomonella* (L.)

(Brunner, 1993). It is the most important parasitoid of CM and is considered as the natural enemy with the highest potential in integrated management of this pest (Lacey and Unruh, 2005). *Ascogaster quadridentata* is a strong flier and good searcher, its life cycle is synchronized with that of CM (Brunner, 1993). Adult females lay their eggs in CM eggs and larvae develop during the egg and larval stages of the host (Shaw and Wallis, 2014).

Diapause represents a syndrome of developmental, physiological, biochemical and behavioural attributes that together serve to enhance survival during seasons of environmental adversity (Denlinger, 2003; Kumar *et al.*, 2015). CM diapause as mature 5th-instar larvae (Brown, 1991; Neven, 2012). CM diapause has been experimentally induced and successfully terminated in laboratory colonies (Singh and Ashby, 1986; Ashby and Singh, 1990). In a CM control program using the sterile insect technique, diapause was used to store larvae during off season as a tool to accumulate them to achieve good over flooding

Handling Editor: Yaghoub Fathipour

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Received: 2 April 2018, Accepted: 3 November 2019
Published online: 9 December 2019

ratios during peak spring adult flight, by releasing large numbers of sterilized moths in a short time (Bloem *et al.*, 1997). In the laboratory, diapausing CM larvae can survive for up to two years (Singh and Ashby, 1986). This experiment examines the possibility of storing *A. quadridentata* inside artificially enhanced diapausing CM larvae using a similar technique of previous experiments related to storing CM. It also evaluates quality characteristics of *A. quadridentata* stored inside CM larvae under diapause and standard conditions. Storing the parasitoid for the suitable time and releasing it in the field in a large scale will contribute to a successful biological control program against CM.

Materials and Methods

Insect cultures

CM larvae used in this study were obtained from a laboratory colony that had been reared for over 80 generations on an artificial diet (Mansour and Mohamad, 2004). The colony originated from moths collected from several locations near the city of Damascus, Syria, in the summer of 1995. Males from natural population were introduced into the colony every summer to maintain vigour of the colony ((Bloem *et al.*, 2004). Transparent plastic trays (19 × 14 × 5 cm) each containing about 800 g of larval rearing diet were used for rearing CM larvae. Trays were incubated under a 16L: 8D cycle, 27 ± 2 °C, and humidity of 75 ± 5% (RH). The emerged adults were placed in an oviposition cage similar to that reported by Proverps and Logan (1970). Females deposited their eggs on wax paper sheet wrapped around the oviposition cage.

The parasitoid, *A. quadridentata*, colony originated from CM larvae collected from orchards in the apple production area north of Damascus. Parasitoid adults (wasps) were maintained in plexiglass containers (parasitism containers) (40 × 30 × 20 cm) under the same condition as the CM colony. Sheets of wax paper (12 × 16 cm) carrying CM eggs (egg

sheets) were fastened to the inside wall of the parasitism containers using hooks. Twenty-four h. later, the egg sheets were removed and placed above the larval rearing trays that contained CM diet. One week later, the egg sheets were removed and the plastic trays were incubated under the same environmental conditions as for the CM colony. After 4 weeks, the trays were transferred into a wooden cabinet (Mohamad *et al.*, 2015) to collect parasitoid adults.

Treatments

Four parasitism containers each containing 20 pairs (males and females) of the parasitoid adults (24-48 h. old) were used. Five egg sheets (11 × 13 cm), each carrying about 300-350 eggs (24-48 h. old) were fastened to the walls of each container. The containers were incubated at 25 ± 2 °C, 16L: 8D and 75 ± 5% RH to obtain the parasitized CM eggs. Twenty other egg sheets, similar to that mentioned before, were fastened to the walls of parasitoid containers free of parasitoids to obtain non parasitized CM eggs. All egg sheets (40 sheets) were removed 24 h. later and placed each above a tray (40 trays containing artificial CM diet) as the following: Ten parasitized egg sheets (treatment 1 or T1) and 10 non parasitized egg sheets (T2) were placed above 20 trays (10 replicate/T), and trays were incubated under diapause conditions. Similarly, 10 parasitized egg sheets (T3) and 10 non parasitized egg sheets (T4) were placed above 20 trays (10 replicate/T) and trays were incubated under standard conditions for CM rearing (25 ± 2 °C, 16L:8D and 75 ± 5% RH) (T3 + T4 were control treatments). The egg sheets for T3 and T4 were removed 7 days later, examined under a stereo-microscope and the number of hatched eggs was recorded. The trays were covered with muslin to prevent the larvae from escaping and incubated under same previous environmental conditions. Four weeks later, the muslin was removed and the trays were transferred into the emergence cabinet (27 ± 2 °C, 16L: 8D,

35 ± 5% RH). The emerged *A. quadridentata* and CM adults were collected daily, counted and their number was recorded.

Induction and termination of diapause

The trays of (T1 and T2) were incubated at 25 ± 2 °C, 50 ± 5% RH and 12L: 12D cycle. One week later the egg sheets were removed from above the trays, examined under a stereomicroscope, and percentage egg hatch was calculated. Corrugated cardboard rolls (18 × 13 × 4 cm) were placed vertically on the top of each tray at day 18 to collect diapausing larvae and the trays were covered with muslin. The cardboard rolls containing diapausing larvae were removed from the trays on day 30, labelled appropriately, placed in black polyethylene bags and stored in a cold room at 15 ± 2 °C, 0L: 24D cycle, 50 ± 5% RH for 100 days. The bags were then transported to a refrigerator at 0 ± 2 °C, 0L: 24D, 50 ± 5% and stored for 50 days. Before terminating diapause, ¼ cardboard roll from each tray (3 trays/T) was carefully cut. Each quarter roll was examined, diapausing and dead larvae were counted and their number was recorded. After examination, the quarters were returned to their original cardboard rolls. The cardboards were then transferred to the emergence cabinet at 27 ± 2 °C, 16L: 8D cycle and 35 ± 5% RH to break diapause. Emerged *A. quadridentata* and CM adults were collected daily, counted and their number was recorded. At the end of emergence, 3 cardboard rolls/T were selected at random, shredded, examined and the number of dead insects (larvae or pupae) were recorded.

Assessment of wasp quality

Samples of emerged wasps from diapausing CM larvae were compared with others from non-diapausing larvae (standard conditions) and the following parameters were used for comparison.

Weight: 10 males and 10 females/replicate were weighed using an electrical balance (Sartorius, d = 0.01 mg) (wasps were chilled before weighing). The weight of each wasp was

recorded and means weigh/wasp was calculated.

Longevity: wasps (10 males and 10 females/replicate) 1-24 h. old were placed individually in small transparent plastic cups (6 × 3.5 cm) with muslin lids, provided with 75% honey solution as fine droplets on the inside walls of the cups. The cups were incubated at 25 ± 2 °C, 16L: 8D, 70 ± 5% RH. The wasps were checked daily, dead insects were removed, their number was recorded and longevity was calculated.

Parasitism rate: 10 pairs of wasps (male and female) per replicate were placed in Petri dishes provided with water-moistened cotton wicks (one pair/dish, 8 cm). One egg sheet (5 × 5 cm)/pair carrying 50 CM eggs (24-48 h old) were daily exposed to the females for five successive days. Forty eggs were used to evaluate parasitism rate during the first 5 days of exposure (Nakama and Foester, 2001). The dishes were incubated at 25 ± 2 °C, 16L: 8D, 60 ± 5% RH. Five days later, the eggs were examined for parasitism (Luis and Doetzer, 2006). The eggs were covered with a thin layer of paraffin oil to, more easily, distinguish parasitized eggs under a stereomicroscope. The number of parasitized eggs/female was recorded and percentage of parasitized eggs was calculated.

Statistical analyses

Data was subjected to analyses of variance (ANOVA). Means were separated by Fisher's protected least significant difference (LSD) test (StatView. Abacus Concepts Inc., Barkeley, California). All experiments were replicated 3 times.

Results and Discussion

Results on the effects of the standard and diapause rearing conditions on *A. quadridentata* are given in Table 1. The table shows that diapause conditions did not significantly affect egg hatch from the host ($P > 0.05$). Most of the data previously reported indicated that the egg-larval endoparasitoids

do not affect egg hatch of their hosts (Brown *et al.*, 1988; Brown and Friedlander, 1995; Delury, 1998). Ashby and Singh (1990), Bloem *et al.* (1997) and Bloem *et al.* (2005) found also that diapause conditions did not affect CM egg hatch. Mean number of hatched eggs/tray under standard condition was 271.1 and 267.2 for parasitized and non-parasitized eggs, respectively. Under diapause condition, the mean number of hatched eggs/tray was 269.2 and 267.2 for parasitized and non-parasitized eggs, respectively.

Results clearly show that the standard conditions did not allow any larva to enter diapause. The number of larvae under diapause conditions were about 130 larvae originated from non-parasitized eggs, and increased significantly ($P < 0.05$) to become around 142 larva resulted from parasitized eggs. The increase in egg hatching from parasitized eggs is mostly due to endoparasitism, since some studies have shown that some parasitoids, inside their hosts, may produce some hormones that help the host to survive until the parasitoids themselves complete their life cycle. These findings are consistent with results reported by several others before (Beckage, 1986; Reed-Larsen and Brown, 1999).

There were no significant differences ($P > 0.05$) under standard conditions, between parasitized and non-parasitized (1.3, 1.1 individual, respectively) in the numbers of dead larvae and pupae. However, their numbers under diapause conditions increased to 9.6 and 9.8 individuals, respectively ($P < 0.05$). Although the mean number of dead larvae and pupae was low, the increase of death may be due to the long storage period that continued for about 6 months.

The results in Table 1 also show that the mean number of CM adults under standard conditions was about 127 moths originated from non-parasitized eggs. The number decreased significantly ($P < 0.05$) to 11 moths in parasitized eggs. This is because, most of parasitized eggs gave adults of *A. quadridentata*. Under diapause conditions, the mean number of moth was about 120 which

resulted from non-parasitized eggs. The mean number also decreased significantly ($P < 0.05$) to 6 moths resulting from parasitized eggs. In general, the mean number of moths under diapause conditions was significantly lower than their numbers under standard conditions whether resulting from parasitized or non-parasitized eggs. This may be due to the length of the storage period.

Results in the same table show that the mean number of *A. quadridentata* that emerged from CM larvae subjected, for 6 months, to diapause conditions was 122.9, while the mean number was about 128.5 under standard conditions. Though there is significant difference ($P < 0.05$) between the two means, it is noticeable that *A. quadridentata* was able to live inside its host in acceptable numbers for 6 months under diapause conditions.

Results of examining specific biological characteristics of *A. quadridentata* adult reared under standard and diapause conditions are summarized in table 2. The results show that host (CM) rearing methods, under (standard or diapause conditions) did not significantly affect the weight of the parasitoid ($P > 0.05$). The mean weight of males was 2.7 and 2.6 mg under standard and diapause conditions, respectively, and for females it was 3.7 and 3.4 mg in the same order. Under both rearing methods, longevity was not significantly affected ($P > 0.05$) between males reared under standard condition (50.6 day) and others reared under diapause conditions (52.1 day). Mean female longevity was similar to that of males, which was around 49.6 and 51.3 day under standard and diapause conditions, respectively. Table 2 also shows percentage of parasitism/female. The data show that percentage of *A. quadridentata* females emerged from CM larvae reared under standard conditions was 86.4% and increased significantly ($P < 0.05$) to 97.8% for females emerged from CM larvae reared under diapause conditions. This increase may be due to stability of physiological factors of parasitoids which were reared through diapause (Corly and Capurro, 2000).

Table 1 Effects of standard and diapause rearing conditions on *Ascogaster quadridentata* in its host, codling moth (CM) *Cydia pomonella*.

Stages	No. of insects / tray of diet (Mean \pm SD)		
	Parasitic condition	Standard conditions	Diapause conditions
Eggs	Non-parasitized	322.8 \pm 32 a	326.2 \pm 33 a
	Parasitized	324.7 \pm 34 a	326.5 \pm 31 a
Egg hatching	Non-parasitized	266.6 \pm 28 a	267.2 \pm 30 a
	Parasitized	271.1 \pm 29 a	269.2 \pm 29 a
Diapausing larvae	Non-parasitized	0.0 a	129.8 \pm 8.0 b
	Parasitized	0.0 a	141.9 \pm 11.0b
Dead CM larvae and pupae	Non-parasitized	1.1 \pm 0.3 a	9.6 \pm 2.3 b
	Parasitized	1.3 \pm 0.2 a	9.8 \pm 3.1 b
CM adults	Non-parasitized	126.9 \pm 8.0 a	120.2 \pm 7.0 b
	Parasitized	11.1 \pm 1.5 a	6.1 \pm 1.1 b
<i>A. quadridentata</i> adults	Non-parasitized	0.0 a	0.0 a
	Parasitized	128.5 \pm 9.0a	122.9 \pm 9.0b

The means followed by the same letter in each row are not significantly different ($P < 0.05$)

Table 2 Some quality characters of *Ascogaster quadridentata* adult reared on standard and diapause conditions in its host *Cydia pomonella*.

Rearing conditions	Wight (mg) (Mean \pm SE)		Longevity (day) (Mean \pm SE)		%Parasitism $\frac{\text{♀}}{\text{♂}}$ /(Mean \pm SE)
	♂	♀	♂	♀	
Standard	2.7 \pm 0.1 a	3.7 \pm 0.1 a	50.6 \pm 5.0 a	49.6 \pm 4.0 a	86.4 \pm 3.0 b
Diapause	2.6 \pm 0.1 a	3.4 \pm 0.1 a	52.1 \pm 4.0 a	51.3 \pm 5.0 a	97.8 \pm 4.0 a

The means followed by the same letter in each column are not significantly different ($P < 0.05$).

Based on the results of this study, the parasitoid storage method by diapause induction to store *A. quadridentata* in its host can be used for several months. This makes it feasible to mass rear and accumulate large numbers of this parasitoid, during fall and winter seasons. Stored parasitoid can be released in the field when needed in the spring or summer, increasing the efficiency of a biological control program against CM.

Acknowledgments

I would like to thank Dr. M. Mansour and Dr. A. Ramadhan for their very helpful and insightful comments on the manuscript. I also thank Dr. I. Othman, Director General of Syrian Atomic Energy Commission and Dr. F. Kurd-

Ali Head of the Agricultural Department, for their support of this research.

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ذخیره پارازیتوئید تخم-لارو *Ascogaster quadridentata* (Hym: Braconidae) در داخل لاروهای میزبان خود کرم سیب *Cydia pomonella* در شرایط دیابوز

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دریافت: ۱۳ فروردین ۱۳۹۷؛ پذیرش: ۱۲ آبان ۱۳۹۸

چکیده: پارازیتوئید تخم-لارو *Ascogaster quadridentata* Wesmael (Hym: Braconidae) به مدت شش ماه در لاروهای دیابوزی سن آخر کرم سیب *Cydia pomonella* (L.) (Lep: Tortricidae) ذخیره شد. درصد خروج حشرات کامل زنبورهای پارازیتوئید از لاروهای دیابوزی حدود ۸۶/۴ درصد بود. ویژگی‌های بیولوژیکی (وزن و طول عمر) برای هر دو زنبور پارازیتوئید که در لاروهای غیردیابوزی (شرایط استاندارد) و دیابوزی کرم سیب پرورش یافته بودند مشابه بود. داده‌ها نشان داد که درصد زنبورهای پارازیتوئید ماده حاصل از شرایط استاندارد ۸۶/۴ درصد بود، در حالی که زنبورهای پارازیتوئید ماده خارج شده از لاروهای دیابوزی به طور معنی داری به ۹۷/۸ درصد افزایش یافت. این یافته‌ها ممکن است به پرورش انبوه زنبور پارازیتوئید *A. quadridentata* کمک نماید.

واژگان کلیدی: کرم سیب، کنترل بیولوژیکی، ذخیره زنبور پارازیتوئید