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Diapause in the egg parasitoid *Trichogramma cordubensis*: role of temperature

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Abstract

The role of temperature in the induction of diapause in *Trichogramma cordubensis* (Hymenoptera: Trichogrammatidae), under controlled laboratory conditions, was investigated using *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs as hosts. Results indicate that prestorage temperatures and the duration of exposure of the parasitoids to these temperatures affected the induction of diapause. It was possible to induce diapause in prepupae of *T. cordubensis* by exposing the preimaginal stages (prior to the prepupal stage) to 10°C for at least 30 days, but adults emerged without diapause when the duration of exposure was of only 10 or 20 days. Parasitoids failed to enter diapause when prestorage temperatures were 7 or 12°C, regardless of the duration of exposure. However, at these two temperatures, preimaginal development of *T. cordubensis* was delayed, allowing short-term storage (40 days at induction temperatures followed by 30 days at 3°C) by keeping parasitoids in quiescence without reducing the percentages of adult emergence. Good percentages of adult emergence after long-term low-temperature storage (30 or 40 days at 10°C followed by six months at 3°C) occurred only when *T. cordubensis* was in diapause. The long-term storage of parasitoids in diapause allows an enlargement in the mass rearing potentialities of this species for future biological control releases by allowing producers to stockpile the parasitoids for release in the field season.

Keywords: Trichogramma; Diapause induction; Quiescence; Temperature; Storage

1. Introduction

In the life cycle of many insects, the orderly process of development can be interrupted by a period of dormancy, which results in protecting a vulnerable stage from adverse environmental conditions. Dormancy in *Trichogramma* spp. may be through either quiescence (López and Morrison, 1980; Keller, 1986; Rossi, 1993) or diapause (Boivin, 1994; Rossi, 1997). Quiescence is halted or slowed development as a direct response to unfavourable environmental conditions, with development resuming immediately after favourable conditions return (Saunders, 1982; Chapman, 1998). A period of developmental arrest combined with adaptive physiological changes, with development resuming not necessarily on return of suitable conditions, is called diapause (Blum, 1985; Chapman, 1998). Diapause is not a direct response to unfavourable conditions, since it is often induced by cues that predict the advent of unfavourable season. These cues are not by themselves favourable or unfavourable for growth or reproduction but serve as a trigger to synchronize the life cycle with environmental conditions (Saunders, 1982; Blum, 1985; Chapman, 1998).

Some species of the genus *Trichogramma* Westwood (Hymenoptera: Trichogrammatidae) enter diapause in the prepupal stage and temperature is considered to be the key factor inducing diapause during preimaginal development (Pizzol, 1978; Anunciada, 1983; Voegelé et al., 1986; Zaslavski and Umarova, 1990). The photoperiod is also a reliable physical cue for the induction of diapause, but according to Zaslavski and Umarova (1982, 1990), changes in daylength only triggers diapause in the progeny of *Trichogramma evenescens* through a maternal influence.

Trichogramma cordubensis Vargas and Cabello is a native thelytokous species of São Miguel Island (Azores)

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(Pintureau et al., 1991). Its effectiveness as a biological control agent for agricultural pests existing in the Azores islands has been investigated in our laboratory by studying parasitoid biology (Garcia and Tavares, 1995, 1997; Garcia et al., 1995a, 2001), population dynamics (Garcia et al., 1995b), and rearing techniques (Tavares and Vieira, 1992).

Most of the research on diapause in *Trichogramma* spp. concerns developmental arrest under natural environments (Burbutis et al., 1976; Voegelé, 1976; López and Morrison, 1980; Anunciada, 1983; Keller, 1986; Rossi, 1993). Pizzol (1978) primarily performed studies concerning artificially induced diapause in *T. evanescens* (which became *Trichogramma maidis*), followed by the studies of Voegelé et al. (1986), Zaslavski and Umarova (1982, 1990) and Laing and Corrigan (1995), but there are no reports on the occurrence of diapause in *T. cordubensis*, either under natural or artificially controlled environments.

Controlling quiescence or diapause induction in egg parasitoids could increase the efficiency of both short and long-term storage of *Trichogramma* spp., with important implications for their use as biological control agents (Voegelé et al., 1986; Hawlitzky and Voegelé, 1991; Boivin, 1994). Hence, the purposes of the present work are to determine the role of temperature and duration of exposure on the induction of diapause and/or quiescence in *T. cordubensis* in the laboratory, and to evaluate the length of time that the parasitoids can be stored at low temperature with a high percentage of adult emergence after storage.

2. Material and methods

2.1. Insects

The strain of *T. cordubensis* used in this experiment was originally collected from parasitized eggs of *Autographa gamma* L. (Lepidoptera: Noctuidae) found at Ribeira do Guilherme (São Miguel island, Azores) in August 1995. Parasitoids have been reared in the laboratory, at 20 ± 1°C, 75 ± 5% r.h. and L16:D8, for approximately 65 generations on *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs, according to the methods of Tavares and Vieira (1992). In view of the fact that *T. cordubensis* is one of the two strictly thelytokous species in the genus *Trichogramma* (Silva and Stouthamer, 1996), there are no problems related to deterioration of stock due to inbreeding during the continuous rearing of this species.

2.2. Experimental design

Eggs of *E. kuehniella* were presented to wasps on egg cards. These egg cards were prepared by spraying a fixed area (1×0.8cm) of an index card with a water solution of non-toxic glue, and then spreading host eggs on this surface (500 ± 10 eggs). The host eggs were less than 24 h old and have previously been irradiated with ultraviolet light for 20 min to prevent development (Voegelé et al., 1974). Each egg card was exposed to five unmated *T. cordubensis* females (less than 24 h old), inside a glass tube (7×1cm) with a drop of honey solution (10%) to provide the parasitoid with a carbohydrate source. After exposure to parasitoids (24 h at 25 ± 0.5°C, 75±5% r.h. and L16:D8), egg cards were assigned to each of the three different prestorage temperatures (7 ± 0.5, 10 ± 0.5 and 12 ± 0.5°C), for 0 (no exposure to prestorage temperatures), 10, 20, 30 or 40 days. Eggs were exposed in environmental chambers at 75 ± 5% r.h. and L8:D16. According to Pizzol (1978), *Trichogramma* diapauses under short daylength. Therefore, we used a photoperiod of L8:D16 for the prestorage of the parasitoids. Moreover, the chosen short daylength corresponds to the photoperiod observed in the Azores (36°55′43″S and 39°43′23″N) during the winter season (Beck, 1968). Thirty-five egg cards were randomly assigned to each period of exposure at the respective prestorage temperature. After exposure, these egg cards were placed in an environmental chamber under a storage temperature of 3°C, 75 ± 5% r.h. and in full darkness, for a period of 0 (no storage), 30, 60, 90 or 180 days. Subsequent to each period of storage at 3°C, five egg cards (i.e. replicates) were transferred to an environmental chamber at 25 ± 0.5°C, 75 ± 5% r.h. and 16L:8D until emergence of the adults. This was made by using environmental chambers at steeply increasing temperatures (6, 13 and 20 °C), holding the egg cards 24 h in each chamber. For each egg card the percentage of adult emergence was estimated by dividing the number of blackened host eggs (i.e. parasitized eggs) with emergence holes by the total number of blackened eggs, using a dissecting microscope at 25×.

The two egg cards remaining in each treatment were also removed subsequent to each period of storage at 3°C to determine the preimaginal stages of the parasitoid. This was done in order to observe if preimaginal development was arrested after the exposure of the parasitoids to low prestorage temperatures and/or storage at 3°C. For this, dissections were performed using the Toluidine blue method described in Pizzol (1978). After eclosion the embryo is released into the host egg but stays surrounded only by the embryonic exuvia, therefore this ‘preimaginal stage’ is called the ‘free-living embryo’ (Volkoff et al., 1995). The mature larvae were identified by the presence of well-developed salivary glands, nervous chain and mandibles, as well as by the rudiments of imaginal disks (Pizzol, 1978; Saakian-Baranova, 1990; Volkoff et al., 1995). The prepupae were identified by the disappearance of the salivary gland and the presence of well-developed imaginal disks of legs, wings and antennae, and also by the presence of scattered urate bodies (Pizzol, 1978; Anunciada, 1983; Volkoff et al., 1995; Dahlan and Gordh, 1996, 1997). The pupal stage was identified by the presence of distinct legs, wings, antennae, body segmentation, genitalia and compound eyes (Anunciada, 1983; Dahlan and Gordh, 1996, 1997). As the pupa matures, the colour of the eyes changes from white to dark red, allowing the distinction between white-, pink- and red-eye pupae (Anunciada, 1983; Volkoff et al., 1995). The preimaginal stages of the parasitoids stored at 3°C for 30 days after exposure to low prestorage temperatures were not studied because at this temperature the preimaginal development of the parasitoid is very slow. Consequently, after 30 days, parasitoids will most probably be at the same preimaginal stage that they were at the time of their transfer to the temperature of storage.

Parasitized host eggs that were subjected neither to low prestorage temperatures nor to the 3°C storage temperature continuously developed at 25 ± 0.5°C, 75 ± 5% r.h. and 16L:8D (control treatment). For this treatment, preimaginal stages of the parasitoids were not examined.

Finally, the mean number of parasitoids in the prepupal stage, after storage at 3°C and without previous exposure to low prestorage temperatures was determined to evaluate the amount of time that parasitoids can be stored at 3°C without reducing the number of parasitoids that develop to prepupae in comparison with parasitoids that continuously developed at 25°C.

2.3. Data analysis

Percentage of adult emergence and the mean number of parasitoids in the prepupal stage after storage at 3°C were first estimated with their standard errors.

A three-way ANOVA was used to study the effects of prestorage temperature, duration of the prestorage temperature and period of storage at 3°C, and their interactions, on the percentage of adult emergence. A one-way ANOVA was conducted on data regarding the number of parasitoids in the prepupal stage after storage at 3°C (during zero, 30 and 60 days), without previous exposure to low prestorage temperatures. When statistical differences existed between data sets ($P < 0.05$), Fisher's Protected Least Significant Difference tests (PLSD) were used to separate differing means. To reduce variance differences, data concerning the percentages of adult emergence were transformed by arcsine$\sqrt{(x)}$ and the numbers of parasitoids in the prepupal stage were transformed by $\sqrt{(x + 0.5)}$, before analyses of variance were performed (Zar, 1996). All analyses were performed using SPSS 10.0 Windows (SPSS Inc., 1999).

3. Results

The three-way ANOVA analysis showed that the percentage of adult emergence of *T. cordubensis* was significantly affected by the prestorage temperature ($F = 47.54$, df = 2 and 260, $P < 0.0001$), the duration of exposure to the prestorage temperature ($F = 49.33$, df = 4 and 260, $P < 0.0001$), the period of storage at 3°C ($F = 51.63$, df = 4 and 260, $P < 0.0001$), and all the possible interactions between these three factors (all $P < 0.0001$). The percentages of adult emergence at the prestorage temperatures of 7 and 12°C decreased significantly ($P < 0.05$, PLSD tests) with an increase in the duration of storage at 3°C (Fig. 1A and C). At 10°C, this tendency was only observed for prestorage exposure times of 10 and 20 days. At this temperature and for longer exposure periods (i.e. 30 and 40 days), the per-

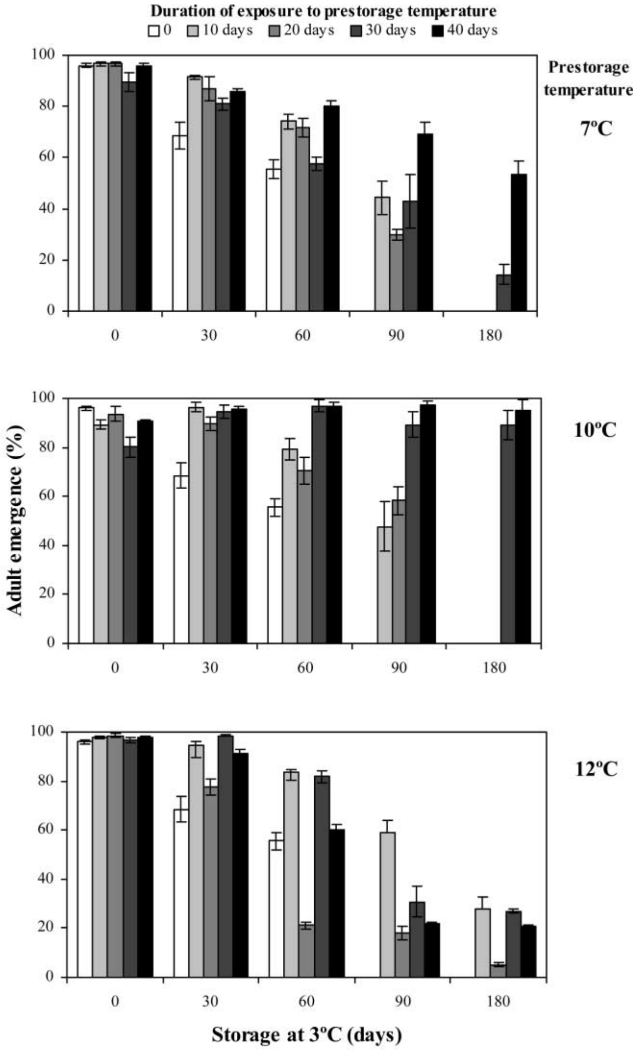

Fig. 1. Mean percentage of adult emergence (±s.e.) of *T. cordubensis* after no or several periods (i.e. 10, 20, 30 and 40 days) of exposure to: (A) 7°C; (B) 10°C; and (C)12°C, followed by storage at 3°C for 0, 30, 60, 90 and 180 days.

centages of emergence remained fairly high, with values similar to the control treatment (Fig. 1B). Results in Fig. 1A show that more than 50% of the parasitoids emerged after they have been exposed to 7°C for 40 days and subsequently stored at 3°C for 90 and 180 days. Yet, percentages of emergence differed significantly from the control treatment ($P < 0.05$, PLSD tests). Some parasitoids emerged after 180 days at 3°C following exposure to 12°C for 10 and 20 days (contrary to what was observed at 7 and 10 °C); however, the percentages were extremely low (Fig. 1C).

Parasitoids that did not experience low prestorage temperatures can only be stored at 3°C for a maximum of 60 days, because adult emergence does not occur afterwards (Fig. 1A–C). However, in parasitoids that were stored at 3°C without any prestorage treatment, there were significantly fewer parasitoids at the prepupal stage than in the wasps that developed continuously at 25°C (43.2 ± 8.4 and 20.0 ± 4.2 after 30 and 60 days of storage, respectively, and 97.4 ± 8.8 for the control treatment) ($F = 25.515$, df = 2 and 12, $P < 0.0001$, one-way ANOVA). These results show that the preimaginal development of the majority of wasps aborts before the prepupal stage, when parasitoids are transferred to 3°C during their embryonic development. After a period of 90 and 180 days of storage at 3°C, the preimaginal development of all parasitoids is aborted before the prepupal stage.

Results in Table 1 show that the preimaginal development of *T. cordubensis* was not arrested when parasitoids were kept at 7 and 12 °C during the period of induction. At these temperatures, and as time of storage increased, the preimaginal development of wasps went either up to adult emergence without any interruption at a particular stage, or parasitoids failed to emerge from the hosts. Nevertheless, when parasitoids emerged from the host, the preimaginal development was delayed, indicating that parasitoids were in quiescence. Similar results were observed when parasitoids were held at 10°C for only 10 or 20 days. In contrast, the development of parasitoids that were held at 10°C for at least 30 days was arrested in the prepupal stage, indicating that wasps entered diapause, tolerating storage at 3°C for a period achieving 180 days (Table 1).

4. Discussion

Anunciada (1983) and Voegelé et al. (1986) pointed out that *Trichogramma* spp. exhibit four types of preimaginal development corresponding to differences in the length of time for emergence of adults to occur: continuous, partial, arrested and aborted developments. In continuous preimaginal development, all wasps develop until emergence of adults without any interruption. Partial preimaginal development occurs when one fraction of the population develops continuously and the other enters diapause. Arrested preimaginal development happens when all parasitoids of a population enter diapause. Finally, the preimaginal development is considered to be aborted when it ceases in an earlier stage, previous to the prepupa, resulting in death.

Our results show that it is possible either to arrest or to delay the preimaginal development of *T. cordubensis* by inducing diapause or quiescence with different low prestorage temperatures and periods of exposure. In our study *T. cordubensis* appears to have diapaused as a prepupa, but quiescence seems to have occurred in all the preimaginal stages. Among insects, including *Trichogramma*, diapause generally occurs in a specific stage (Zaslavski and Umarova, 1990; Rossi, 1993). Rossi (1993) demonstrated that quiescence in *T. cacoeciae* and *T. evanescens* could occur in the egg, larval, prepupal and pupal stages.

T. cordubensis quiescence was observed when parasitoids were exposed to prestorage temperatures of 7 and 12°C from 10 to 40 days, with subsequent storage at 3°C. Under these conditions, parasitoids could be stored at low-temperature for a period of 60 days. However, the percentages of *T. cordubensis* emergence appeared to decrease with an increase in the duration of storage at low-temperature. Therefore, under such conditions, wasps should not be exposed to 3°C for a period exceeding 30 days. Jalali and Singh (1992) verified that several *Trichogramma* species could be stored as prepupae for short term (for 49 days) at 10 °C when eggs of *Corcyra cephalonica* (Stainton) (Lepidoptera: Galleriidae) are used as hosts.

When parasitoids were transferred to 3°C during their embryonic development, the number of wasps that survived after exposure to storage decreased noticeably, resulting in a decrease in the number of parasitoids in the prepupal stage or in an abortion of *T. cordubensis* preimaginal development. López and Morrison (1980) also observed that preimaginal stages of *T. pretiosum* prior to the prepupa are less tolerant to low temperatures. In contrast, Rossi (1993) found that, when the egg and larval stages of *T. evanescens* and *T. cacoeciae* experienced temperatures below 5 °C for a short time, adult emergence remained unaffected.

Induction of diapause in *T. cordubensis* was accomplished by submitting preimaginal stages prior to the prepupal stage to 10 °C for at least 30 days. At this temperature, and for shorter periods of exposure, either parasitoids failed to enter diapause or died inside the host egg. These findings lead us to suggest that a minimum conditioning period (30 days) and a precise temperature regime (10°C) during a particular preimaginal stage (prior to the prepupal stage), are necessary to induce diapause in the prepupae of *T. cordubensis*. Nevertheless, it is likely that some of the parasitoids that have been exposed to 7°C for 40 days and subsequently

Table 1
Preimaginal stages of *T. cordubensis* observed at three prestorage temperatures, after different periods of prestorage and storage at 3°C (0, 60, 90 and 180 days). LAR—larvae; PRE—prepupae; PUW—pupae (white-eye); PUP—pupae (pink-eye); PUR—pupae (red-eye)

Duration of prestorage (days)	Duration of storage at 3°C (days)											
	Prestorage at 7°C				Prestorage at 10°C				Prestorage at 12°C			
	0	60	90	180	0	60	90	180	0	60	90	180
10	LAR	PRE	PRE[a]	PRE[b]	LAR	PRE	PRE[a]	PRE[b]	LAR	PRE	PRE	PRE, PUW[a]
20	LAR	PRE	PRE[a]	PRE, PUW, PUR[b]	PRE	PRE, PUW	PRE[a]	PRE[b]	PRE, PUW	PRE, PUW, PUP[a]	PUP[a]	PUP[a]
30	LAR	PRE	PRE[a]	PRE[a]	PRE	PRE	PRE	PRE	PUR	PUR	PUR[a]	PUR[a]
40	PRE	PRE	PRE	PRE	PRE	PRE	PRE	PRE	PUR	PUR	PUR[a]	PUR[a]

[a] Only a few adults emerged.

[b] No adults emerged (see Fig. 1A–C).

stored at 3°C for 90 and 180 days were in diapause, since more than 50% still emerged. However, we must not disregard the fact that the percentages of emergence were significantly lower than in the control. Diapause induction results in an increase in parasitoid emergence after exposure to storage at low temperature. Obviously, the parasitoids exposed to 10°C for only 10 or 20 days were not sufficiently conditioned or did not develop sufficiently to enable them to survive under the low temperature of storage. Likewise, the prestorage temperature of 7°C for 40 days appears to have induced diapause only in one fraction of the population, while the other portion of parasitoids failed to emerge from the hosts. The temperature that induces diapause in *T. cordubensis* is very close to the lower threshold temperature (i.e. 10.4 °C) of this species (Garcia, 2001). Anunciada (1983) and Rossi (1993) also found, for other species of *Trichogramma* (*T. evanescens*, *T. japonicum*, *T. maidis* and *T. cacoeciae*), that temperatures near the parasitoids' lower thresholds can induce diapause. Zaslavski and Umarova (1990) demonstrated that holding prepupae of *T. semblidis*, *T. pintoi*, *T. evanescens*, *T. embryophagum* and *T. principium* at 12.5 and 15 °C is a key factor in inducing diapause in these species. Moreover, Rossi (1993) verified that, under natural conditions, diapause in *T. evanescens* and *T. cacoeciae* can be induced by temperatures between 7 and 10 °C, acting during a certain time on a particular preimaginal stage (prior to the prepupa), as it was observed in the present work for *T. cordubensis*, but under controlled conditions. In accordance with this author, if unfavourable conditions occur during the egg or larval stages (i.e. during non-diapausing stages), either development is continuous or the parasitoid dies. Similarly, if low temperatures continue after the breaking of diapause, parasitoid mortality will be high, since the only developmental stage that is resistant to low temperatures is the prepupa.

On the other hand, works regarding the role of photoperiodic cues for the induction of diapause during the preimaginal development of *Trichogramma* failed to reveal any accurate effect. The work of Maslennikova (1959) with *T. evanescens* showed that at 10°C about 95–97% of the parasitoids entered diapause regardless of the photoperiod. Yet, at 15°C, the number of parasitoids that entered diapause increased with decrease of the photoperiod. Later Zaslavski and Umarova (1990) demonstrated for *T. principium* that 100% of the parasitoids entered diapause when held at 12.5°C at short daylength, regardless of the photoperiod experienced by the parental generation. However, at 15°C, a higher number of parasitoids in diapause were observed when the parents experienced a shorter daylength. The works from Maslennikova (1959) and Zaslavski and Umarova (1990) point to a possible role of photoperiod in the induction of diapause during preimaginal development, depending on the temperature. In such cases, short day enhanced the tendency to diapause in the progeny. Our results showed that 10°C is a suitable prestorage temperature for induction of diapause in *T. cordubensis*, although some diapause induction may have occurred at 7°C. At this last temperature, below the lower threshold temperature of *T. cordubensis*, it is possible that the short daylength might have had an important role in determining the occurrence of diapause. Therefore, future experiments need to be performed to study the possible effects of photoperiod in the induction of diapause during the preimaginal development of *T. cordubensis*, particularly at prestorage temperatures differing from the species lower threshold temperature.

Understanding the mechanisms of diapause induction under laboratory conditions in *T. cordubensis* enables long-term storage of this insect, allowing producers to stockpile the parasitoids for release in the field season. In turn, a reduction in the number of generations necessary for mass rearing the parasitoid may help to prevent alterations in the acceptance of the natural host due to the continuous rearing of the wasp in a factitious host. However, when inducing diapause in *Trichogramma* spp., one must not disregard other biological traits of post-diapausing insects because poor-quality parasitoids could yield low efficiency in biological control programs (Cerutti and Bigler, 1995; Pompanon and Boulétreau, 1997). Therefore, experiments are now being performed to study the effects of diapause on additional traits, such as wasp longevity and fecundity.

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