Influence of cold storage on survival and fitness of *Mastrus ridens*, an ectoparasitoid of codling moth

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Abstract The effects of cold storage on eclosion, longevity and fecundity of adults of *Mastrus ridens*, an ectoparasitoid of codling moth, were investigated by holding different life-stages (eggs, developing larvae, mature larvae, pre-pupae and pupae) at 4°C for different periods (4, 6, 8, 10 and 12 weeks). Cold storage of eggs for 4 weeks resulted in high larval mortality. The development periods after cold storage decreased and the immature mortality increased with advancing developmental stages before cold storage. The longevity of females decreased with increasing cold storage of pre-pupae and pupae. Neither fecundity nor oviposition period of females emerging from the seven selected treatments was different from the control (females reared at optimum temperature). Results indicated that 7- to 10-day-old *M. ridens* larvae can be stored for up to 12 weeks at 4°C without any detrimental effect on their survival or quality of adulthood. Application of this finding will help with supply of the large numbers of parasitoids needed during the release programme.

Keywords *Mastrus ridens*, biological control, codling moth, cold storage, mass production.

INTRODUCTION

Mastrus ridens (Horstmann) (Hymenoptera: Ichneumonidae) is an arrhenotokous, synovigenic, gregarious, idiobiont ectoparasitoid of cocooned codling moth (CM) (*Cydia pomonella* (L.) (Lepidoptera: Tortricidae)) larvae (Mills 2005; Devotto et al. 2010; Sandanayaka et al. 2011). Eggs (about 4 per CM larva) are laid on the codling moth larva within its cocoon and the parasitoid larvae consume their host, spin their own cocoons, pupate and eclose within the codling moth cocoon (Sandanayaka et al. 2011; Charles et al. 2013). The mean duration of the immature parasitoid (egg to mature larvae) was 15.5 days at ca 21°C (Charles et al. 2013) and, when held at 25°C, adult *M. ridens* females and males lived for 18.8±3.6 and 17.2±5.2 days respectively (Devotto et al. 2010).

As part of a programme to establish *M. ridens* in apple-growing areas as a biocontrol agent of CM, adult parasitoids are released to coincide with the seasonal appearance of wild, cocooning codling moth larvae – typically from mid January to late April throughout much of New Zealand where codling moth is univoltine. During this period, about 5000 healthy adult *M. ridens* per week are required for releases and colony maintenance. Low-temperature storage of parasitoids can allow greater flexibility in production, and synchronise production in the laboratory with the timing of release in the field (van Lenteren & Tommasini 2003; van Lenteren 2003; Colinet & Boivin 2011). However, prolonged storage at low temperatures may also be associated with deformation (Tezze

& Botto 2004), poor immature survival, reduced adult longevity, reproduction and performance, and increased mortality (Rundle et al. 2004; Luczynski et al. 2007; Sajid et al. 2010; Silva et al. 2013).

The present study investigated the feasibility of storing immature stages at 4°C (a temperature well below their development threshold) for different periods to help manage the supply of large numbers of adult *M. ridens* during the release period.

MATERIALS AND METHODS

Sixty mated female *M. ridens* (2–7 days old) were placed in a vented plastic container (21×20×20 cm) with honey-agar diet and water at 22±0.5°C and 16 h photoperiod. A corrugated cardboard roll containing 50 diapausing codling moth larvae (CM roll) was exposed to those females for 48 h in order to produce an estimated 100 parasitoids/roll. Forty CM rolls were exposed this way and then held under these environmental conditions in 4 groups of 10 for a further 5, 8, 11 and 13 days to deliver *M. ridens* middle- and late-instar larvae, pre-pupae and pupae after total development periods of 7, 10, 13 and 15 days respectively (A. Chhagan, unpublished data). The 10 rolls with each life-stage were then moved to a climate chamber under natural daylight (through the glass door) at 4°C and ca 60% RH, and two rolls held for each of five different periods (4, 6, 8, 10 and 12 weeks) (Table 1). An additional two CM rolls were exposed to *M. ridens* females for 24 h as above (to provide eggs) and then stored at 4°C for 4 weeks. Two CM rolls containing developing parasitoids were held at 22±0.5°C and a 16 h photoperiod as the controls.

Following the cold-storage periods, the CM rolls were returned to the climate chamber at 22±0.5°C and 16 h photoperiod for surviving

parasitoids to complete their development. They were examined regularly for parasitoid pupae, which were removed from the corrugated cardboard rolls and held in a Petri dish inside a vented plastic container (15 × 15 × 20 cm) with a supply of honey-agar diet and water for emerging adults. Two weeks after the last adult emerged, numbers of dead parasitoid pupae were counted. The times taken for eclosion after removal from cold storage and the total numbers of surviving adults were compared between treatments.

Adult longevity

The longevity of adults was measured by pairing between 18 and 20 males and females that emerged within 1–3 days of each other from each treatment (Table 2). Each pair was confined in an 11 × 4 cm vented plastic vial containing honey-agar and water, and were not exposed to CM larvae. The humidity inside the vials was maintained with a layer of moist paper towel at the base of the vial. Adult survival was recorded daily by 9:00 am. Honey-agar and water in the vials were refreshed frequently until both adults died.

Oviposition by treated females

Oviposition by a subsample of 12 females that emerged from seven selected treatments (young larvae 7d-4w, 7d-8w, 7d-12w, mature larvae 10d-6w, 10d-12w, pre-pupae 13d-4w, pupae 15d-4w) and the controls was measured. Newly emerged females and males from the same treatment were confined in a vented plastic container (15 × 15 × 20 cm) for 2–3 days for mating. Each female was then moved to an individual vented plastic vial (11 × 4 cm) containing honey-agar diet and water and provided with three diapausing CM larvae cocooned within white tissue paper

Table 1 Abbreviated names of the treatments tested in cold storage on performance of *Mastrus ridens*.

Pre-treatment periods at 22±0.5°C	Storage period at 4°C				
	4 weeks	6 weeks	8 weeks	10 weeks	12 weeks
24 h (eggs)	24h-4w				
7 days (young larvae)	7d-4w	7d-6w	7d-8w	7d-10w	7d-12w
10 days (mature larvae)	10d-4w	10d-6w	10d-8w	10d-10w	10d-12w
13 days (pre-pupae)	13d-4w	13d-6w	13d-8w	13d-10w	13d-12w
15 days (pupae)	15d-4w	15d-6w	15d-8w	15d-10w	15d-12w

every Monday, Wednesday and Friday until she died. The numbers of eggs or 1st instar larvae of *M. ridens* found on each parasitized CM larva were recorded. The life-time realised fecundity, oviposition period and longevity of the females in each treatment were compared with those of the 'control' females. The eggs and 1st instar larvae, which were not disturbed during counting, were held at $22\pm0.5^{\circ}\text{C}$ and the sex of adults recorded.

Statistical analysis

Gaussian Generalized Linear Models (GLMs) (Dobson 1990) were used to compare the various responses to combinations of pre-storage development and storage times. The GLM function in R version 3.1.2 (R Core Team 2014), which is an implementation described by Hastie & Pregibon (1992), was used to calculate the probabilities of obtaining the observed results under the null hypothesis of no difference from the control.

RESULTS

Post-treatment development and survival to adults

The lowest survival was recorded from the 24h-4w treatment, from which only 24 larvae developed to pupae and emerged as adults. Only a single parasitoid pupa per CM larva developed in this treatment. Dead 1st instar *M. ridens* larvae were found on 84% of CM larvae after 4 weeks of cold storage. In the other treatments, when *M. ridens* larvae survived to pupae, their feeding activity on a codling moth larva destroyed evidence of any other *M. ridens* larvae that had died during (or before) the treatments. This meant that the original numbers of *M. ridens* eggs laid, and hence the treatment mortalities, could not be directly determined. However, treatment survival was estimated from the numbers of adults emerged from *M. ridens* pupae collected.

The time taken for *M. ridens* larvae or pupae to complete their development at $22\pm0.5^{\circ}\text{C}$ after cold storage decreased with increasing age of the insects at the time of treatment. However, within an age group, the cold-storage period did not make a significant difference to the time required for adult eclosion (Table 2). The next lowest

survival was recorded in pupae in the 15d-12w treatment (Table 2). Except for 90.6% survival in 13d-8w treatment, survival in all treatments with 13- and 15-day development stages decreased with increasing storage period.

In addition to direct mortality effects, some of the adults that emerged from the 13d-10w, 13d-12w, 15d-10w and 15d-12w treatments had deformed wings and ovipositors, indicating negative effects of prolonged cold storage on their mobility and reproduction.

Adult longevity

The longevity of between 18 and 20 pairs was measured, except for the 15d-12w treatment from which only 12 pairs emerged. Too few adults emerged from the 24h-4w treatment to be included in the longevity test.

Female longevity was either equal to or higher than male longevity except for 13d-10w, 13d-12w and 15d-10w treatments. Regardless of the storage periods, the females emerging from 13d (pre-pupa) and 15d (pupa) development stages lived for significantly shorter periods than the females emerging from the controls ($P<0.01$) (Table 2). The longevity of the females emerging from the other treatments did not differ from that of the females emerging from the controls ($P>0.01$). Males emerging from 13d-12w treatment lived for significantly shorter periods than those from the controls, while males emerging from 7d-8w and 10d-10w lived for significantly longer than those from the controls ($P<0.01$). The longevity of males emerging from the remaining treatments was not significantly different from that of males emerging from the controls ($P>0.01$) (Table 2).

Oviposition by treated females

The realised fecundity (estimated from the total number of eggs laid), oviposition period and the lifespan of 11 to 12 females that survived the seven selected treatments were not significantly different from those of the females that emerged from the controls ($P>0.01$) (Table 3). Both females and males emerged from eggs and 1st instar larvae (remaining after the fecundity assessment) confirming the fertility of males.

Table 2 Mean ($\pm$ SE) post-storage development period (days), pupal survival (%) and mean ($\pm$ SE) female and male longevity (days) of *Mastrus ridens* at $22\pm0.5^\circ\text{C}$ after different storage periods at 4°C . Mean values with asterisks in female longevity and male longevity columns are significantly different ($P<0.01$) from the longevity of control females and males (compared by Gaussian Generalized Linear Models).

Treatment	Post-storage development period (n ¹)	Pupal survival	Female longevity (n)	Male longevity (n)
young larvae - 4w	17.0 $\pm$ 0.2 (156)	96.3	17.9 $\pm$ 1.4 (19)	11.5 $\pm$ 0.7 (19)
young larvae - 6w	17.1 $\pm$ 0.2 (138)	96.5	15.5 $\pm$ 0.9 (18)	11.6 $\pm$ 0.6 (18)
young larvae - 8w	17.3 $\pm$ 0.2 (158)	95.8	15.9 $\pm$ 1.1 (20)	12.5 $\pm$ 0.9* (20)
young larvae - 10w	17.8 $\pm$ 0.2 (211)	93.4	13.2 $\pm$ 0.9 (20)	9.5 $\pm$ 0.5 (20)
young larvae - 12w	18.7 $\pm$ 0.3 (160)	93.6	15.1 $\pm$ 0.9 (20)	9.8 $\pm$ 0.5 (20)
mature larvae - 4w	13.4 $\pm$ 0.2 (96)	95.0	13.2 $\pm$ 1 (18)	10.4 $\pm$ 1 (18)
mature larvae - 6w	13.6 $\pm$ 0.1 (257)	97.4	14.6 $\pm$ 1 (18)	11.8 $\pm$ 0.7 (18)
mature larvae - 8w	15.4 $\pm$ 0.2 (76)	90.5	13.5 $\pm$ 0.4 (19)	10.9 $\pm$ 0.6 (19)
mature larvae - 10w	14.1 $\pm$ 0.5 (119)	93.7	15.9 $\pm$ 1.1 (20)	12.6 $\pm$ 0.9* (20)
mature larvae - 12w	13.6 $\pm$ 0.3 (103)	96.3	16.7 $\pm$ 1.2 (20)	10.7 $\pm$ 0.9 (20)
pre-pupae - 4w	8.8 $\pm$ 0.2 (139)	88.5	12.9 $\pm$ 0.8* (18)	10.4 $\pm$ 0.7 (18)
pre-pupae - 6w	9.2 $\pm$ 0.2 (202)	85.6	12.9 $\pm$ 0.9* (18)	11.6 $\pm$ 1 (18)
pre-pupae - 8w	8 $\pm$ 0.2 (173)	90.6	12.3 $\pm$ 0.7* (18)	10.9 $\pm$ 0.7 (18)
pre-pupae - 10w	7.5 $\pm$ 0.2 (152)	86.9	8.9 $\pm$ 0.6* (19)	9.5 $\pm$ 0.7 (19)
pre-pupae - 12w	7.6 $\pm$ 0.5 (67)	72.8	5.1 $\pm$ 0.7* (18)	6.6 $\pm$ 0.6* (18)
pupae - 4w	7.4 $\pm$ 0.3 (90)	84.9	12.5 $\pm$ 0.6* (20)	9 $\pm$ 0.6 (20)
pupae - 6w	6.8 $\pm$ 0.2 (100)	86.2	11.2 $\pm$ 0.6* (18)	11.4 $\pm$ 0.8 (18)
pupae - 8w	4.7 $\pm$ 0.2 (83)	87.4	10.6 $\pm$ 0.7* (18)	10.2 $\pm$ 0.8 (18)
pupae - 10w	5.8 $\pm$ 0.4 (55)	55.6	8.5 $\pm$ 0.7* (18)	9.3 $\pm$ 0.7 (18)
pupae - 12w	3.7 $\pm$ 0.2 (46)	47.4	9.8 $\pm$ 0.8* (12)	9 $\pm$ 0.9 (12)
Control		95.3	16.4 $\pm$ 1.3 (19)	9.6 $\pm$ 0.6 (19)

¹Number of parasitoid pupae.

Table 3 Mean ($\pm$ SE) of estimated fecundity (total number of eggs laid), oviposition period (days) and the life span (days) of the selected females emerged from seven selected treatments and the control.

Treatment (n)	Estimated fecundity	Oviposition period	Female lifespan
young larvae - 4w (12)	45.6 $\pm$ 4.4	15.4 $\pm$ 2	27.4 $\pm$ 0.7
young larvae - 8w (12)	61.4 $\pm$ 4.2	12.8 $\pm$ 0.7	18.7 $\pm$ 1.1
young larvae - 12w (12)	54.3 $\pm$ 5.5	14.7 $\pm$ 2.1	24.1 $\pm$ 3.3
mature larvae - 6w (12)	50.8 $\pm$ 4	12.3 $\pm$ 1.6	27.5 $\pm$ 3.2
mature larvae - 12w (12)	39.0 $\pm$ 3.9	10.3 $\pm$ 0.7	20.2 $\pm$ 2.3
pre-pupae - 4w (12)	61.0 $\pm$ 6.7	19.2 $\pm$ 1.9	27.4 $\pm$ 3.3
pupae - 4w (12)	60.7 $\pm$ 8.4	15.2 $\pm$ 2.2	24.0 $\pm$ 2.6
Control (11)	58.5 $\pm$ 4.5	14.3 $\pm$ 0.6	27.2 $\pm$ 1.2

DISCUSSION

The cold-storage options of *M. ridens* development stages were investigated in order to meet the demand for large numbers of healthy adults for field releases. The aim was to determine the threshold storage period at 4°C that did not reduce the quality of *M. ridens* adults emerging from stored developing stages. The results indicate that 7- to 10-day-old *M. ridens* larvae can be stored for up to 12 weeks at 4°C without any detrimental effect on their survival or quality of adulthood. Pre-pupae and pupae were more sensitive to extended periods of cold storage, with lower survival during storage and reduced longevity of emerging adults. These trends are similar to those reported for other parasitoid species. For example, *Diaeretiella rapae* mummies stored at 5±1°C suffered 50% mortality after 40 days (Silva et al. 2013) and there was high mortality of late pupal stages of *Encarsia formosa* after storage at 4°C and 7°C, with the detrimental effects of cold increasing as the temperature was lowered and the duration of storage extended (Luczynski et al. 2006). Hackermann et al. (2008) reported that *Hyssopus pallidus*, an ectoparasitoid of CM, could be cold stored for up to 14 days without any negative effect on its parasitism and reproductive capacity.

In optimum cold-storage conditions, insects enter a dormant state known as quiescence, resulting in a slowdown of their metabolic activities. When removed from short periods of storage they develop and behave normally (Leopold 1998). In this study the shortest storage period tested was 4 weeks. Short-term cold storage (less than a week) of pre-pupae and pupae may not have a high impact on the fitness of emerging adults. The deformation of adults emerging from *M. ridens* pre-pupae and pupae after 10–12 weeks of cold storage period may have resulted from slow metabolic activities and embryonic development during storage at a critical period of metamorphosis. Tezze & Botto (2004) reported that the proportion of malformed adult *Trichogramma nerudai* increased significantly with increase of storage period at 4 ± 1°C.

The low survival of *M. ridens* eggs stored at 4°C for 4 weeks meant that they were not

exposed to longer storage periods. The 24 adults that survived from that treatment were all healthy, took an average 27.5 days to develop at 22±0.5°C after the cold storage. Devotto et al. (2010) reported that the development time from egg to adult of *M. ridens* at 25°C was 24 ± 2 days. Therefore, it was assumed that the eggs surviving for 4 weeks in cold storage proceeded to develop at a normal rate at 22±0.5°C. No pupal mortality was recorded from this treatment. However, dead 1st instar *M. ridens* larvae were found on 84% of CM larvae in that treatment, suggesting that the 1st instar larvae were not cold tolerant and died soon after emergence.

Most of the females emerging from 13d-12w and 15d-12w treatments had deformed ovipositors. Preliminary observations revealed that these females were unable to oviposit. Based on the data collected from the seven treatments it appears that storage at 4°C had no negative effect on oviposition by females developing from larvae stored for up to 12 weeks or from pupae stored at 4°C for 4 weeks. Males emerging from all seven treatments successfully fertilised females.

Female longevity under host-deprived conditions may reflect their survival capacity in the field in low host populations. Bai & Smith (1993) reported a positive correlation between reproduction and survival of *Trichogramma minutum*. Female *M. ridens* lived longer when they were provided with hosts for oviposition (Table 3) than when they were provided only with honey-agar and water (Table 2). The reasons for this were not clear, although host-feeding (Sandanayaka et al. 2011) may have made a difference. A female *M. ridens* emerges with about five mature eggs and achieves about 17 mature eggs after 3–4 days of host deprivation (Hougardy et al. 2005; Hougardy & Mills 2006). The longevity of *M. ridens* females emerged from cold-stored larvae did not differ from the longevity of the females reared at 22±0.5°C, suggesting that cold storage may not have a negative effect on larvae. Devotto et al. (2010) reported that female *M. ridens* lived longer than males only at 15°C, when longevity of adults were tested at 15°C, 25°C and 35°C. The present results showed that the

female longevity was longer than male longevity when reared at $22\pm0.5^{\circ}\text{C}$ and did not change after the cold storage of larvae. Cold exposure of *M. ridens* larvae (7–10 days development before cold storage) for 12 weeks did not influence longevity or morphological malformations of adults and reproductive capacity of females.

In summary, chilling *M. ridens* larvae for 4–12 weeks did not affect pupal survival or adult longevity and may be an option to manage the supply of parasitoids during release periods. However, chilling other life-stages is often detrimental. For example, relatively long periods of chilling (10 or 12 weeks) pre-pupae and pupae led to low pupal survival relative to shorter chilling periods, and to deformed adults and reduced adult longevity.

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