Effect of Cold Storage on Emergence, Longevity, Fertility, and Survival of *Cotesia marginiventris* (Hymenoptera: Braconidae)[1,2]

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Abstract The effect of short-term cold storage on non-diapausing pupae and adults of *Cotesia marginiventris* (Cresson) was evaluated for developing protocols for the mass production of this parasitoid. When stored at 10°C, successful emergence of adults from cocoons occurred within 6 d and was not affected by storage time (0 d, 4 d, 12 d, or 20 d). Longevity of males and females was not affected by cold storage, but fewer males and females remained alive per consecutive 8 d interval after emergence at 27°C. A fertility experiment revealed that fewer progeny (F_1 cocoons, F_1 females) were produced by parental females previously cold-stored as pupae in cocoons for 20 d rather than 4 d. A within-storage adult survival experiment demonstrated that males and females were not affected by any of the storage times (8 d, 16 d, 24 d, 32 d). This survival rate was achieved by removing the adults from the cold at 8 d intervals, placing them at 23 ± 1°C for ~ 2 h and providing honey. Another fertility experiment revealed that more F_1 males, but fewer F_1 females, were produced when the parental females (which had been cold-stored as adults) were stored for 32 d rather than for 16 d. This study suggests that adults are more amenable to short-term storage than pupae if the adults are periodically removed for feeding. Nevertheless, cold storage of cocoons (pupae) for 20 d or adults for 32 d can limit the production of female progeny.

Key Words Cold storage, mass rearing, parasitoids, *Cotesia marginiventris,* fertility

Cotesia marginiventris (Cresson) is an important solitary endoparasitoid of lepidopterous pests in agroecosystems (McCutcheon 1987, Ruberson et al. 1994, Cecilia and Luna 1996). It also has been used as a model organism for studies on multitrophic interactions between crop plants, pests and their natural enemies (Turlings et al. 1990, Farmer 1997). Augmentation of *C. marginiventris* as a biological control strategy may be feasible in the near future. The Biological Control and Mass Rearing Research Unit (BCMRRU), ARS, USDA is developing a semi-automatic system for the efficient mass production of this larval endoparasitoid using the beet armyworm, *Spodoptera exigua* (Hübner), as host. The development of cold storage techniques may greatly enhance the efficacy of this developing rearing system.

The biological control industry needs efficient techniques for storing natural enemies (Glenister and Hoffmann 1998). Gilkeson (1990) stated that the commercial

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[2]This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or a recommendation by USDA for its use.

industry could benefit from short-term storage, because the producer could balance the differences between product supply and demand; whereas, long-term storage could reduce production costs if insects were only reared during the crop growing season, then stored during other seasons. Cold storage has several perceived advantages. These include (1) synchronization of natural enemy abundance with host/prey abundance, (2) shipment of natural enemies over long distances without high mortality from starvation, and (3) prolongation of natural enemy life span by a reduction in metabolism.

Previous research on cold storage of hymenopterous parasitoids has considered species that parasitize aphid nymphs and adults (Archer et al. 1973, Hofsvang and Hågvar 1977, Whitaker-Deerberg et al. 1994, Bueno and van Cleave 1997, Waggoner et al. 1997), bruchid beetle mature larvae and pupae (Hanna 1935), lepidopterous eggs (Gautam 1986), lepidopterous larvae (Jayanth and Nagarkatti 1985, Jalali et al. 1990, Okine et al. 1996), lepidopterous pupae (Lasota and Kok 1986, McDonald and Kok 1990), lygus bug eggs (Jackson 1986), muscid fly pupae (De Bach 1943), and whitefly nymphs (Lacey et al. 1999).

Jalali et al. (1990) were the first to consider cold-storing *C. marginiventris* as part of a project assessing the use of *C. marginiventris* to suppress populations of *Spodoptera litura* (F.) in Bangalore, India. They found that freshly-formed cocoons could be stored at 10°C for 20 d with only 19% of adults not emerging from cocoons, which did not differ significantly from the percentage (15%) not emerging from non-stored cocoons. Longevity of females that successfully emerged from cocoons after storage was 6 d after 10 storage days, 5 d after 20 storage days, and 3 d after 30 storage days at 5°C. The within-storage mortality of adult females was 59% for 30 d and 82% for 35 d in storage at 10°C. Males were more susceptible to cold than were females; 92% of the males died when held in storage for 35 d. Adult fecundity was reduced when parasitoids were stored for more than 20 d at 5°C; and when stored for longer periods, progeny sex ratio was male-biased (Jalali et al. 1990).

In this present study, the issue of short-term cold storage of non-diapausing *C. marginiventris* pupae (in cocoons) and adults was revisited. The objectives were (1) to estimate the emergence rate, longevity and fertility of cold-stored pupae (in cocoons), and (2) to estimate the within-storage survival rate and fertility of cold-stored adults.

Materials and Methods

Parasitoid and host colonies. *Cotesia marginiventris* and the beet armyworm, *S. exigua,* were from colonies at the BCMRRU, ARS, USDA, Mississippi State, MS. Host larvae were reared on an agar soybean flour-wheat germ meridic diet (after King and Hartley 1985). Meridic diet and *S. exigua* eggs were added to 32-cell trays by a partially automated system (see Tillman et al. 1997). In 4 d, the first to second instars were removed from the original trays and moved by hand to 32-cell trays containing fresh diet, at a density of ~5 larvae per cell. Before sealing all cells, the larvae were exposed to mated 1 to 2 d old female *C. marginiventris* for 24 h in a 18.9 L clear plastic 'sting box' (45.7 × 66.0 × 8.9 cm, W × L × H) with lid. After parasitization, *S. exigua* larvae in cells per tray were placed on rackveyors (see Smith and Nordlund 1999) in an environmentally controlled rearing room (at 27°C, 60-70% RH, 16:8 (L:D) h photoperiod). By the ninth day after parasitization, *C. marginiventris* mature larvae had exited hosts and spun cocoons on the sides or lids of the cells. On the tenth day,

cocoons (containing pupae) were harvested by gently pulling them off of the sides or lid of each rearing cell with featherweight forceps. Batches of 200 to 250 cocoons were usually placed together in a single medium-sized Petri dish (15 mm depth × 100 mm diam), labeled with the parasitization and harvest dates on the lid. Some 3,000 or more *C. marginiventris* cocoons have been routinely produced and harvested every 2 wk. The parasitoid has been in production at BCMRRU for >50 continuous generations, the host for >150 continuous generations.

Emergence and longevity of adults stored as pupae. The emergence rate and adult life span of adults previously stored as pupae (inside cocoons) for 0 d, 4 d, 12 d, or 20 d at 10°C was determined. Sample pupae (inside cocoons) were harvested from the laboratory colony on day 10. Dissections of cocoons revealed that by day 9, 10% of the cocoons contained prepupae and 90% contained pupae (n = 50); by day 10, 100% contained pupae (n = 59) (unpubl. data).

Two trials of this experiment were performed. In the first, 40 cocoons were randomly assigned to each of the three storage time treatments, and 100 cocoons were assigned to the control (0 d), for a total of 220 cocoons. In the second trial, 80 cocoons were randomly assigned to each of the three storage treatments, and 73 cocoons were assigned to the control, for a total of 313 cocoons. All cocoons for each storage time treatment were placed inside a medium-sized Petri dish (15 mm × 100 mm) with lid and then stored in a counter top refrigerator in complete darkness at 10°C, 61% RH (median). The control cocoons were placed inside a Petri dish without lid and then positioned directly at the base of a polypropylene rearing cage (30 × 30 × 30 cm, 24 mesh size, Bug Dorm 1™, MegaView Science Education Services Co., Ltd., Taiching, Taiwan), inside an environmentally-controlled rearing room, at 27°C, 60 to 70% RH, 16:8 (L:D) h cycle. Cold-stored cocoons also were placed inside separate polypropylene rearing cages in the same rearing room when removed from storage.

Adults had continuous access to sterile water via moistened cotton balls in a Petri dish base at the bottom of each cage. In addition, the interior of each cage was misted with sterile water each day. Fine droplets of pure bee honey (USDA grade A, Southern Home, Birmingham, AL) were made available on a strip of parafilm hanging vertically from the top of each cage.

Male emergence began by the second day after cocoons, inside cages, had been placed in the rearing room. Female emergence began by the third day, and all adults had emerged by the sixth day, irrespective of days in cold storage (unpubl. data). Adult longevity was calculated from the cumulative proportion of either sex expiring at 8 d intervals. The proportion of cohorts alive (1 minus the proportion expired) was recorded at 8, 16, 24, and 32 d from the date that emergence was first noticed.

Fertility of adults previously stored as pupae. Fertility has been defined as the number of viable progeny produced by an organism (Jervis and Kidd 1996). In this experiment, fertility was represented by the number of F_1 pupae (in cocoons) and emergent F_1 males or females produced by parental females previously cold-stored for 4 d, 12 d, or 20 d at 10°C. This experiment consisted of three trials. For each trial, at least 250 *C. marginiventris* pupae (in cocoons) of a single generation were harvested at random on the tenth day from the 'sting date' (the day that hosts were subjected to parasitization by female *C. marginiventris* in the lab colony) and placed initially inside a medium-sized Petri dish with lid. Petri dishes were then stored at 10°C. At the completion of the required storage time, each Petri dish (without the lid) was placed at the base of a polypropylene rearing cage as described above, then kept inside the rearing room at 27°C, 60 to 70% RH, 16:8 (L:D) h cycle.

After 5 to 7 d from the date that cocoons were removed from cold storage, emergent adults were selected at random from the cages by trapping them inside glass vials (13 mm diam × 100 mm length). Then the open end was stoppered with cotton. While still inside the vials, females were distinguished from males by examining their genitalia with the aid of a binocular microscope.

The total sample size (for each storage time) of the combined trials was 40 (4 d), 35 (12 d), and 38 (20 d) parental females of 2 to 4 d of age. For each storage time, 35 to 40 replicate Petri dishes (trials 1 through 3 combined) were supplied with an ~4 g gelatinous square of meridic diet, then 30 host larvae (late first to second instar *S. exigua*) were placed on or near the diet using featherweight forceps. The lid of the dish was placed over the base as larvae were added to prevent them from escaping.

To facilitate the transfer of female wasps from the vials to the Petri dish arenas, vials were placed for several min on a Perltier Chill Table (BioQuip Products, Gardena, CA). Wasp activity was reduced just enough to prevent any from flying out of any dish before the lid could be re-positioned over the base. Each female parasitoid was gently placed in a dish with featherweight forceps. Dishes were held in a growth chamber at 26.5°C, 65 to 80% RH, 16:8 (L:D) h photoperiod. After 24 h, parental females were removed from each dish. Dishes were checked daily to insure that meridic diet was always available to the larvae; fresh diet was added when necessary. At 10 d after parasitization, all dishes were removed from the growth chamber and placed on the counter top at ambient conditions (23 ± 1°C, 50 to 60% RH) because the F_1 mature larvae had exited their hosts and spun their cocoons on the side, bottom, or lid of the dishes. All of the F_1 cocoons present in each dish were gently removed using featherweight forceps and transferred to clean, small-sized (10 mm depth × 35 mm diam) Petri dishes with lids.

The number of F_1 cocoons produced by each parental female per 30 host larvae per dish was recorded. Only completely formed cocoons were counted. On occasion, parasitoid mature larvae constructed ill-formed cocoons or no cocoon at all. When this happened, the larvae died before metamorphosing into pupae. Successfully emerging progeny were sexed, and the number produced by each parental female was recorded.

Survival rate of stored adults. In this experiment *C. marginiventris* adults were stored at 10°C for 8 d, 16 d, 24 d, or 32 d. Once again, ~250 *C. marginiventris* pupae (in cocoons) of a single generation were harvested at random from the laboratory colony, then placed inside a polypropylene rearing cage and kept inside the rearing room at 27°C, 60-70% RH, 16:8 (L:D) h cycle, for 5 to 7 d. Emergent adult males (3 to 5 d old) and females (2 to 4 d old) were removed from cages at random and placed, individually, into medium-sized Petri dishes with lids. Each Petri dish had four equally-sized compartments (Becton Dickinson Company, Franklin Lakes, NJ); a single wasp was placed in one of the compartments. A water-moistened cotton ball was placed in another compartment to prevent direct contact with the moisture source. The remaining two compartments remained empty. All dishes were placed inside a counter top refrigerator in complete darkness at 10°C, 61% RH.

At 8 d intervals, starting from the date that parasitoids were initially placed in the cold, all dishes were removed and placed on the lab bench. The number of wasps alive in each dish was recorded. Any dead wasps were removed and discarded. Several droplets of a 20% honey water solution were placed inside either of the two empty compartments in each dish. After approximately 2 h, all dishes with living parasitoids were returned to cold storage, if required.

Four trials of this experiment were conducted. The total number of males used in each storage time treatment, for combined trials, was 31 (8 d), 37 (16 d), 39 (24 d), and 32 (32 d); the total number of females was 52 (8 d), 46 (16 d), 44 (24 d), and 51 (32 d). The proportion of male and female cohorts alive per 8 d interval was determined after the onset of emergence at 27°C. The survival rate of males was compared to that of females.

Fertility of previously stored adults. The fertility of adults that had survived cold-storage for either 16 d or 32 d was estimated. A control group of young (1 to 2 d old) parental females of a more recent generation (from cocoons harvested from the laboratory colony, as described previously) were deployed, and their fertility compared to that of the cold-stored females. At the completion of their designated storage time, living males and females were removed from the Petri dishes and placed immediately inside a polypropylene rearing cage and held in the rearing room for 1 to 4 d at 27°C, 60-70% RH, 16:8 (L:D) h cycle. Sterile water and honey were available at all times in the cages.

Two trials of this experiment were performed. In the first trial, medium-sized Petri dishes (15 mm × 100 mm; volume, approximately 80 ml) were deployed, with 10 replicate dishes for the 16 d and 32 d storage times and 20 replicate dishes for the control. All dishes initially contained approximately 4 g of gelatinous meridic diet for the host larvae. In the second trial, the design of the arena was changed because several young host larvae were seen escaping from the Petri dishes. In this trial, plastic 50-ml centrifuge tubes were deployed. A hole (2 cm diam) was bored into the cap of each tube, and the opening was then covered with nylon mesh to provide air circulation. This design made it easier to manipulate host larvae and meridic diet. Approximately 20 ml of meridic diet was poured into the base of each tube. In 30 min, the diet had converted into a gelatinous form. In the second trial, 10 replicate tubes were deployed for 16 d and 32 d storage times, and 10 replicate tubes for the control. Thus, the total sample size for each storage time treatment was 20 parental females per 16 d and 32 d, and 30 parental females per control (0 d).

Thirty *S. exigua* larvae (late first and second instars) were added to each dish or tube using featherweight forceps. Next, a previously cold-stored female parasitoid was added to each dish or tube with featherweight forceps. Dishes or tubes were held in a growth chamber at 26.5°C, 65 to 80% RH, 16:8 (L:D) h cycle. After 24 h, the parasitoids were removed and the dishes or tubes were returned to the growth chamber. Dishes were checked daily to insure that meridic diet was always available to the larvae. There was an excess of diet in the tubes; they did not need to be checked daily. At 10 d after the date that host larvae were exposed to the parasitoid, all dishes and tubes were removed from the growth chamber and placed on the lab bench. The F_1 cocoons were gently removed from the dishes or tubes using featherweight forceps and placed into clean, small-sized (10 mm × 35 mm) Petri dishes with lids.

The data obtained from the first and second trials were combined, because there was no significant difference in any of the measures of fertility between the Petri dish and centrifuge tube designs. The number of F_1 coccons, and emergent males and females produced by each parental female per 30 host larvae per dish or tube was recorded.

Statistical analyses. All data were square-root or arcsine transformed prior to analyses (Sokal and Rohlf 1981). The parametric 2-factor analysis of variance (ANOVA) was used to test the significance of storage time, sex, and the interaction of time and sex on emergence rate and survival rate. It also was used to test the

significance of storage time, days after the onset of emergence, and the interaction between both factors on longevity. When the transformed data did not meet the requirements of normality or homogeneity of variances, nonparametric tests were deployed. The nonparametric 1-factor Kruskal-Wallis ANOVA was used to test the significance of storage time on fertility of adult females previously stored as pupae and the fertility of cold-stored adults. Least square means (2-factor ANOVA) and medians (nonparametric tests) were considered significantly different when $P \leq 0.05$. The Student-Newman-Keuls method or the Dunn's method (Glantz 1992) was used to compare means or medians, respectively, when necessary. All data analyses were performed with Sigma Stat software (1994).

Results

Adult emergence and longevity. The total number of adults emerging from cocoons (per storage time) was 139 (0 d), 93 (4 d), 66 (12 d), and 66 (20 d). The percent emergence of adults did not differ significantly between storage time ($F = 1.22$; df = 3, 8; $P = 0.36$, Fig. 1). Male emergence did not differ significantly from female emergence ($F = 0.61$; df = 1, 8; $P = 0.45$). There was no significant interaction between storage time and sex ($F = 3.15$; df = 3, 8; $P = 0.09$).

Adult longevity of previously stored male pupae was not affected by storage time ($F = 2.19$; df = 3, 16; $P = 0.13$, Fig. 2A), but the proportion of males alive declined progressively from 8 d, 16 d, and 24 d after the onset of emergence ($F = 58.9$; df = 3, 16; $P < 0.0001$). No significant difference was detected between 24 and 32 d

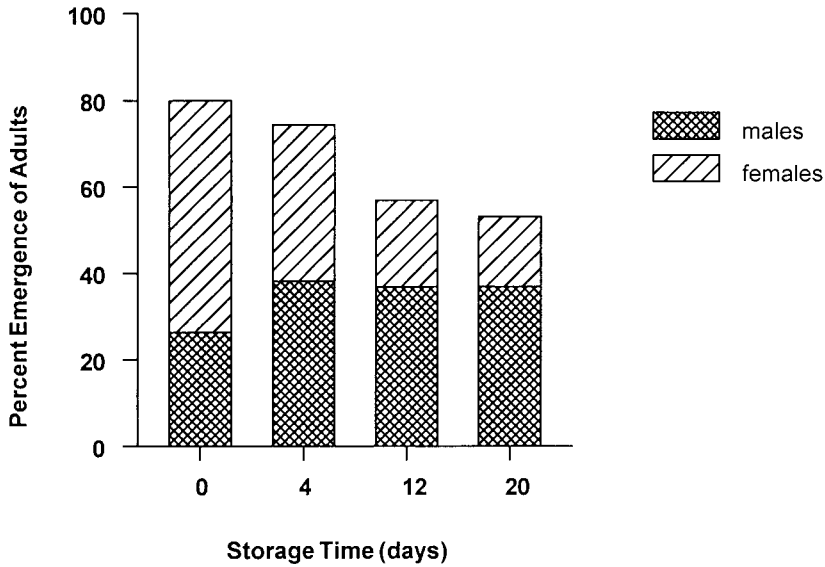

Fig. 1. Least square mean percent emergence of *C. marginiventris* males and females after removal from cold storage. SEM was ± 2.89 for each mean for both sexes (n = 16).

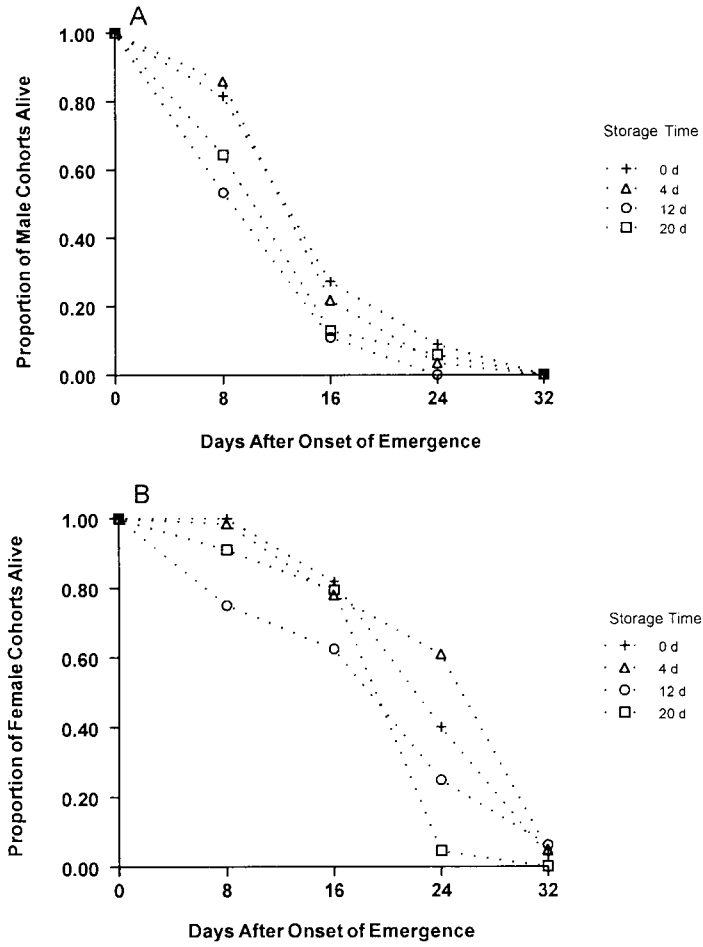

Fig. 2. Least square mean proportion of *C. marginiventris* males (A) and females (B) alive after the onset of emergence. SEM was ± 0.02 for each mean in both graphs (n = 32). Adults were previously cold-stored as pupae per storage time treatments.

intervals. There was no significant interaction between storage time and days after onset of emergence ($F = 0.52$; df = 9, 16; $P = 0.84$). Female longevity was not affected significantly by storage time ($F = 2.96$; df = 3, 16; $P = 0.06$, Fig. 2B), but the proportion of females alive declined progressively from 8 d, 16 d, 24 d, and 32 d after the onset of emergence ($F = 55.2$; df = 3, 16; $P < 0.0001$). There was no interaction between storage time and days after onset of emergence ($F = 1.66$; df = 9, 16; $P = 0.18$). Note that by day 24 (after the onset of emergence), the proportion of males alive was less than 0.10 in all storage treatments; whereas, the proportion of females alive was greater than 0.20 in the 0 d, 4 d, and 12 d storage treatments. The highest proportion

of females alive, by day 24, was 0.61 ± 0.02 (mean ± SEM) in the 4 d storage treatment (Fig. 2B). By day 32, all males and most females were dead.

Fertility of adults stored as pupae. Parental females produced significantly more F_1 pupae (in cocoons) when storage time was 4 d rather than 20 d ($H = 10.01$, df = 2, $P = 0.007$, Fig. 3A). Females stored for 12 d did not produce more F_1 pupae than those stored for 4 d or 20 d. The number of F_1 males that emerged from cocoons did not differ between the three storage time treatments ($H = 3.02$, df = 2, $P = 0.22$, Fig. 3B), but significantly more F_1 females emerged from cocoons in the 4 d than in the 20 d treatment ($H = 19.60$, df = 2, $P < 0.0001$, Fig. 3C).

Survival rate of stored adults. Storage time had no significant effect on the within-storage survival rate of adults when they were removed from storage and fed

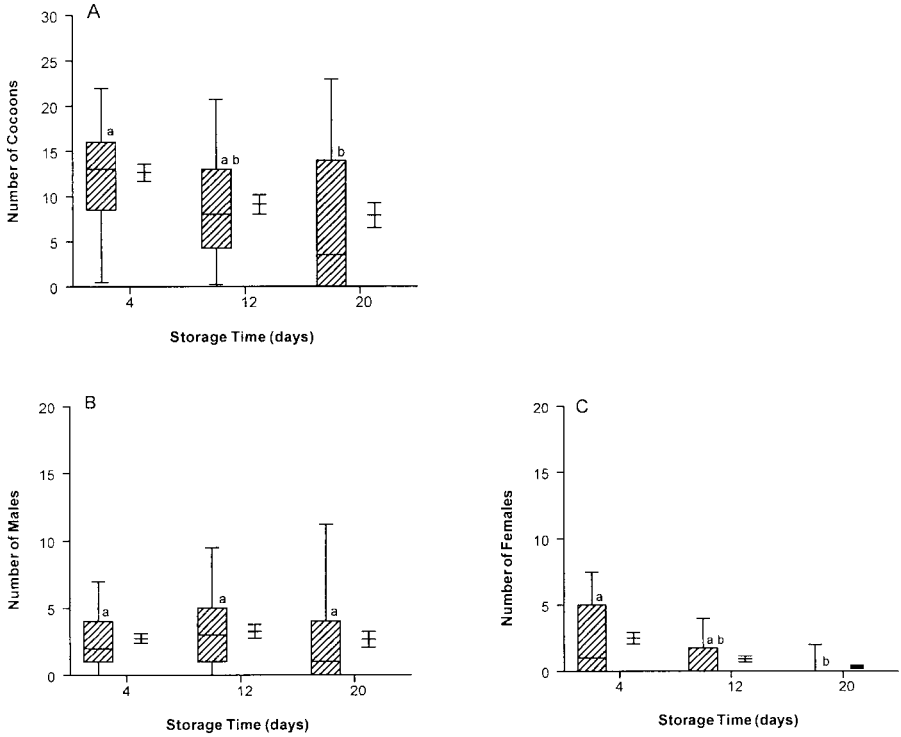

Fig. 3. Median number of F_1 cocoons (A), males (B), and females (C) produced per parental *C. marginiventris* female per 30 *S. exigua* host larvae. Parental females were previously cold-stored as pupae inside cocoons. The whisker boxes represent the median (horizontal line through box), the 25th and 75th percentile points (top and bottom edge of the box), as well as the 5th and 95th percentile points (ends of the vertical lines extending from the edge(s) of each box. The mean ± SEM number of progeny are represented as the small horizontal line, with intersecting error bars, adjacent to the whisker boxes (n = 113).

at 8 d intervals (F = 1.71; df = 3, 24; P = 0.19, Fig. 4). However, survival rate was influenced by sex; female survival was greater than male survival at each storage time treatment (F = 12.26; df = 1, 24; P = 0.002). For example, the mean ± SEM proportion of males alive after 32 d was 0.68 ± 0.03, whereas, the proportion of females alive was 0.95 ± 0.03. The interaction between storage time and sex was not significant (F = 0.10; df = 3, 24; P = 0.96).

Fertility of adult females stored as adults. Parental females produced significantly more F_1 pupae (in cocoons) when storage time was 0 d rather than 32 d (H = 8.12, df = 2, P = 0.02, Fig. 5A). Females stored for 16 d did not produce more F_1 pupae than those stored for 0 d or 32 d. The number of F_1 males that emerged from cocoons did not differ between 0 d and 32 d storage time treatments, although fewer males emerged from cocoons in the 16 d treatment as compared to the 32 d (H = 8.96, df = 2, P = 0.01, Fig. 5B). Significantly more F_1 females emerged from cocoons in the 0 d and 16 d treatments than in the 32 d (H = 13.45, df = 2, P = 0.001, Fig. 5C).

Discussion

The observation that *C. marginiventris* pupae (in cocoons) could be stored for 20 d at 10°C without a reduction in adult emergence was not surprising. Jalali et al. (1990) indicated that *C. marginiventris* cocoons could be stored successfully for 20 d at 10°C, but storage for more than 25 d significantly reduced emergence rates. Sex ratio of progeny from mated females is often male-biased in this species under normal conditions of rearing (Kunnalaca and Mueller 1979, Rajapakse et al. 1992, unpubl.

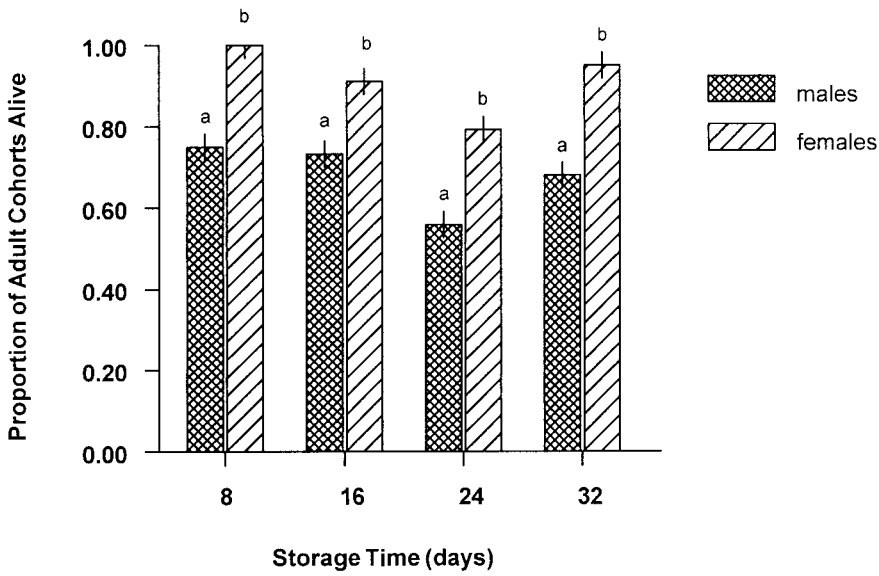

Fig. 4. Least square mean ± SEM proportion of *C. marginiventris* adult cohorts alive in cold storage per time (n = 32).

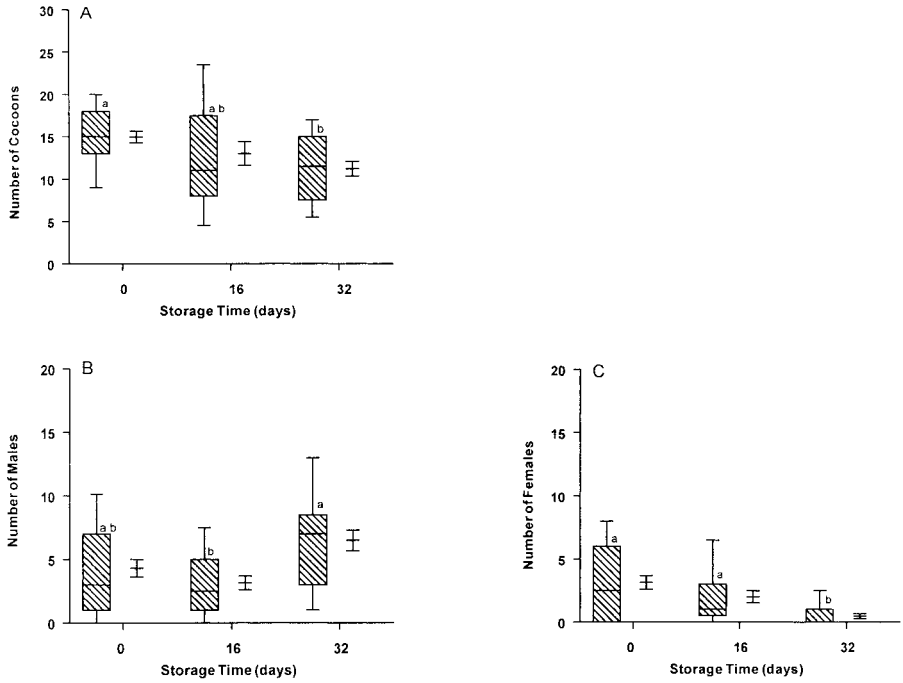

Fig. 5. Median number of F_1 *C. marginiventris* cocoons (A), males (B), and females (C) produced per parental female per 30 *S. exigua* host larvae (n = 70). Parental females were previously cold-stored as adults.

data). Cold storage was shown to have no effect on the emergence rate of either sex in the present study.

Adult longevity was not affected by short-term storage of ≤20 d and the presence of food (honey) inside the rearing cages prolonged the life span of cold-stored and control (non-stored) individuals. For example, after 20 d of storage, >80% of the emergent females were still alive by the sixteenth day after the onset of emergence. In contrast, Jalali et al. (1990) found that the longevity of females was only 5 d after 20 storage days and 3 d after 30 storage days at 5°C. Food was apparently not available to the emergent adults in their study. Johanowitz and Mitchell (2000) reported that non-stored *C. marginiventris* lived from 3 to 33 d (mean = 19 d) in cages that were provisioned with honey at 23 to 30°C.

The survival rate of stored adults was not affected by short-term storage. When adults were removed from the cold and fed at 8 d intervals, females experienced high rates of survival (≥80%) within 32 d at 10°C. Without feeding, only 40% of adult females survived for 30 d (Jalali et al. 1990). The removal and feeding procedure has prolonged the storage potential of other hymenopterous parasitoids (Jayanth and Nagarkatti 1985, Lasota and Kok 1986). For example, adults of the pteromalid *Pteromalus puparum* (L.), a parasitoid of the imported cabbageworm, caged together in groups and supplied with a sugar solution, had survival rates of 80% or more for 30

d at 15°C and 23°C (Lasota and Kok 1986). Periodic removal of adult parasitoids from storage prevents them from starving, because temperatures of ≥10°C will not completely halt parasitoid metabolism. Placement at ambient temperature also permits a brief period of normal respiration.

Cold storage appeared to have a negative effect on the fertility of adult females that were stored as pupae (in cocoons) and as adults. It was evident that by 20 d of storage, parental females previously stored as pupae produced fewer F_1 pupae (in cocoons), and fewer F_1 adult females successfully emerged. Jalali et al. (1990) reported that the fecundity of *C. marginiventris,* stored as cocoons (presumably containing pupae), was significantly reduced after storage for longer than 20 d. Cold storage may deplete the nutrients that mature larvae or pupae have stored during the early larval stages (Flanders 1938). After adult emergence, the reproductive organs of these individuals might not function optimally.

The decline in production of female progeny by cold-stored parental females may have been a result of parasitoid old age, rather than any cold-related physiological stress. Older parasitoid females tend to oviposit fewer female eggs (King 1987). Perhaps, *C. marginiventris* females could not release sperm from their spermathecae, regardless of whether the sperm were viable or not after 32 d in storage. Gerling and Rotary (1974) indicated that the walls of the spermathecal reservoir in *Bracon mellitor* Say thickened as females grew older. Release of sperm became more difficult, such that old females produced progeny that were mostly or completely males.

If cold-stored parental males could not produce viable sperm after being stored for 32 d, the successful insemination of females when placed together in the same rearing cage with these males would not be possible. Also, *C. marginiventris* females might not mate more than once in their lifetime (monandrous). Recent evidence suggests that monandrous species are often solitary, whereas gregarious species are polyandrous (Ridley 1993). In any case, cold storage of adult females for periods of time that reach or exceed the limit of their normal life span at warm temperatures might cause male-biased sex ratios of progeny.

Cold storage might have less impact on the reproductive performance of diapausing individuals than non-diapausing ones (see Tauber et al. 1993, Chang et al. 1996). The diapausing stage of development for *C. marginiventris* has not been reported in the literature, but it is suspected that *C. marginiventris* overwinter as mature larvae (prepupae) in the field in North America. Another solitary *Cotesia* species, namely *Cotesia melanoscela* (Ratzeburg), overwinters as prepupae inside cocoons in North America (Nealis et al. 1996). Induction of diapause in *C. melanoscela* prepupae has been possible in the laboratory (Weseloh 1973).

There is evidence for environmental regulation and geographical adaptation of diapause in parasitoids (Tauber et al. 1986), including *Cotesia* species. Alvi and Momoi (1994) discovered two distinct races of *C. plutellae* in Japan, one race in the north and the other race in the south. Northern populations could be induced into diapause by a short photoperiod, but larvae of the southern populations could not (Alvi and Momoi 1994).

Cotesia marginiventris is native to the West Indies (Muesbeck 1921), but it is also distributed in North America, from Delaware south to Florida, west to Indiana, Kansas, Texas, Wisconsin, Arizona, and California (Marsh 1979). Populations on any of the tropical islands of the West Indies might consist primarily of non-diapausing individuals, because the year-round mild weather might not obligate parasitoids to undergo diapause, at least in response to cold temperatures. In contrast, populations of dia-

pausing individuals would be necessary in the northern-most portions of its range in North America.

Nealls and Bourchier (1995) suggest that inundative releases of the non-diapausing strain of *C. melanoscela* would be more effective than the diapausing strain in suppression of gypsy moth populations. Individuals of the non-diapausing strain emerged more rapidly and had limited exposure to pupal hyperparasitoids. Ballal et al. (1988) indicated that females from a non-diapausing population of *Cotesia kazak* Tel. had a longer adult life span and produced more cocoons than females from a diapausing population.

Perhaps, the use of diapausing *C. marginiventris* would be more appropriate for cold storage over longer periods of time (≥3 months), which could permit the halting of an automatic production system over the winter season. The use of non-diapausing *C. marginiventris* would be suited for short-term storage (≤1.5 months), which could permit temporary halting of the production system for maintenance of machinery, for example. Short-term storage would also facilitate the shipment of non-diapausing *C. marginiventris* to clients for field releases.

In conclusion, the production of healthy, fertile, high-quality insects should be the ultimate goal of any mass rearing program (Nordlund 1998). Cold storage techniques that do not adversely affect the production of female progeny are preferred. This present study suggests that the maintenance of fertile, non-diapausing *C. marginiventris* within rearing systems will be possible if cold storage is limited to 2 to 3 wk for pupae and 4 to 5 wk for adults.

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