Effects of cold storage on adult emergence and fecundity of *Choristoneura occidentalis* (Lepidoptera: Tortricidae)

S.Y. LI and I. S. OTVOS

**PACIFIC FORESTRY CENTRE, CANADIAN FOREST SERVICE,
506 W. BURNSIDE ROAD, VICTORIA, BRITISH COLUMBIA, CANADA V8Z 1M5**

ABSTRACT

The effects of cold storage on *Choristoneura occidentalis* Freeman pupae, in darkness at 2.0 ± 0.5 ^{0}C and 100% R.H. for 0 to 10 weeks, were determined on adult emergence, adult longevity, the number of eggs laid per female, and egg viability in the laboratory. The proportion of adults emerging was not significantly reduced after pupae were stored in the cold room for up to 2 weeks. No adults emerged from pupae being stored for 8 weeks or longer. The lifespan of adult females was longer than that of males. The differences in adult longevity between the two sexes increased after pupae were exposed to cold storage. Cold storage of *C. occidentalis* pupae significantly reduced adult longevity: longer pupal storage resulted in a shorter adult lifespan. After 1 week of cold storage of the pupae, the mean number of eggs laid per female and egg viability were not significantly reduced as compared with those for females not exposed to cold storage as pupae. Egg production and egg viability, however, were significantly reduced when pupae were subjected to cold storage for 2 weeks or longer. Considering all the parameters measured, pupae may be exposed to cold storage for 1 week without deterioration in adult quality.

Key words: *Choristoneura occidentalis*, cold storage, adult longevity, fecundity.

INTRODUCTION

Larvae of the western spruce budworm, *Choristoneura occidentalis* Freeman, undergo obligatory diapause and overwinter in the second larval stage in nature. After about 8 months in diapause, larvae resume development the following spring (Shepherd *et al.* 1995). A non-diapausing colony of *C. occidentalis* was induced in the laboratory, and has been successfully reared on artificial diet since early 1970 (Lyon *et al.* 1972). Compared to a diapausing colony, the non-diapausing colony has a shorter generation time, permitting more rapid adjustments in colony size. This advantage facilitates mass rearing, providing a convenient source of experimental insects for research. Although it is relatively easy to rear *C. occidentalis* on artificial diet in the laboratory, it is time-consuming. Sometimes it is difficult to maintain a large colony to ensure a continuous and qualitatively uniform supply of insects. But there are times when more insects may be produced than can be used immediately. It would be desirable to use these extra insects later if they could be cold stored for a time, without deterioration in their quality.

Cold storage has been used in biological control programs to hold beneficial insects temporarily in the laboratory, to synchronize releases with the development of pest insects in the field, to accumulate sufficient numbers of beneficial insects for field release, or to balance supply and demand in the market (Gilkeson 1990; Bueno and Van-Cleve 1997). Cold storage is also used as a quarantine treatment for some species of insects (Yokoyama and Miller 1989; Toba and Moffitt 1991), and to hold insect cultures temporarily for future research without maintaining continuous rearings. Previous research has shown that cold storage of insects at low temperatures, ranging from 0 ^{0}C to 10 ^{0}C, negatively affects their quality, but to

date, no studies have documented the effects of cold storage on survivorship and fecundity in *C. occidentalis*.

The current study was conducted to determine the effects of cold storage of *C. occidentalis* pupae on adult emergence, adult longevity, the number of eggs laid per female, and egg viability.

MATERIALS AND METHODS

Insects. Pupae used in this study were obtained from a non-diapausing laboratory colony of *C. occidentalis,* which had been reared on artificial diet since 1982. Larvae were reared in groups of five in 20-ml creamer cups on artificial diet modified after Robertson (1979) without formalin, at 20 ± 1 ^{0}C and 55-60% R.H., with a photoperiod of 16:8 (L:D) h. Larvae pupated in the cups and the pupae were collected daily. After collection, the pupae were placed in Petri dishes under the above rearing conditions for 48 h. They were then sexed and 10 male or 10 female pupae were placed in a 170-ml fluted food cup (Sweetheart Cup Co. Inc., Chicago, IL). A total of 550 pupae were thus prepared in 55 cups for each sex. The cups containing 10 pupae each of either males or females were randomly divided into 11 groups. Each group consisted of 10 cups, 5 for each sex. Each cup containing either 10 male or 10 female pupae was used as a replicate. Thus, there were five replicates for each sex in each group. Twenty pupae of each sex in two cups were randomly selected from each group and weighed to the nearest 0.1 mg, using a Sartorius analytical balance.

Effects of Cold Storage on Adult Emergence and Longevity. All pupae were 3 days old before they were stored in darkness at 2.0 ± 0.5 ^{0}C and 100% R.H. for either 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 weeks. For 0 week, the pupae were not cold stored. Following the cold treatment, the pupae were moved to conditions of 20 ± 1 ^{0}C and 55-60% R.H., with a photoperiod of 16:8 (L:D) h. The pupae were checked twice a day for adult emergence. The proportion of males and females emerging was recorded for each replicate. The data were normalized by arcsine-square root transformation, and the transformed data were subjected to a repeated measures analysis of variance to analyze the effects of sex and cold storage duration on adult emergence, using SYSTAT (SPSS Inc. 1996).

To determine the effects of cold storage on adult longevity, freshly emerged adults from each group (50 pupae of each sex) in the above described emergence observation were paired. One pair of adults was placed in a 170-ml cup at 20 ± 1 ^{0}C and 55-60% R.H., with a photoperiod of 16:8 (L:D) h. Up to 20 pairs of adults from each group were set up this way, depending on the number of adults that emerged in a group. Adults were observed twice a day until they died. The longevity data were transformed as $\sqrt{(x + 0.5)}$, where x is longevity in days. The transformed data were subjected to a repeated measures analysis of variance to analyze the effects of sex and cold storage on adult longevity.

Effects of Cold Storage on Adult Fecundity. Female adults used in the longevity observation from each group laid their eggs in the 170-ml cups. The eggs were maintained at 20 ± 1 ^{0}C and 55-60% R.H., with a photoperiod of 16:8 (L:D) h. When the eggs started hatching, the larvae were counted and removed from the cups daily. Unhatched eggs were counted with the aid of a dissecting microscope at 5X magnification. Numbers of hatched and unhatched eggs were recorded for each female. Egg viability was assessed as the percentage of eggs hatching. The data on the total number of eggs laid by a female were transformed as $\sqrt{(x + 0.5)}$, where x is the number of eggs, whereas the data on egg hatching percentage were normalized by arcsine-square root transformation. The transformed data were subjected to a repeated measures analysis of variance to analyze the effects of cold storage on mean number of eggs laid per female and on percentage of egg hatching.

RESULTS

On average, female pupae of *C. occidentalis* weighed 98.6 ± 1.3 mg (± SE, n = 220) with a maximum of 140.4 mg and a minimum of 51.5 mg, and males weighed 66.0 ± 0.9 mg (n = 220) with a maximum of 105.3 mg and a minimum of 26.3 mg. Differences in pupal weight between the two sexes were significant (F = 463.6; df = 1,438; P = 0.0001). There were no significant differences in the pupal weight among the groups of males (F = 1.0; df = 10,209; P = 0.4543), or among the groups of females (F = 1.1; df = 10,209; P = 0.3991), suggesting that group weights, within sex, were equivalent before cold storage.

Effects of Cold Storage on Adult Emergence and Longevity. The proportion of adults emerging was significantly reduced by cold storage (F = 104.3; df = 7,56; P = 0.0001), and was not significantly different between males and females (F = 0.1; df = 1,8; P = 0.7890) (Fig. 1). The percentages of adults emerging (pooled males and females) were 97.0%, 98.0%, and 92.0% after pupae were stored for 0, 1, and 2 weeks, respectively. No significant differences (P > 0.05) in these emergence rates were found. However, after 3 weeks cold storage of pupae, adult emergence was significantly (P < 0.05) reduced. Although adults still emerged after 5 weeks cold storage of pupae, 100% of them were malformed. These malformed adults did not mate, and the females did not lay any eggs (see results below). After 8 weeks cold storage of pupae, no adults emerged (Fig. 1).

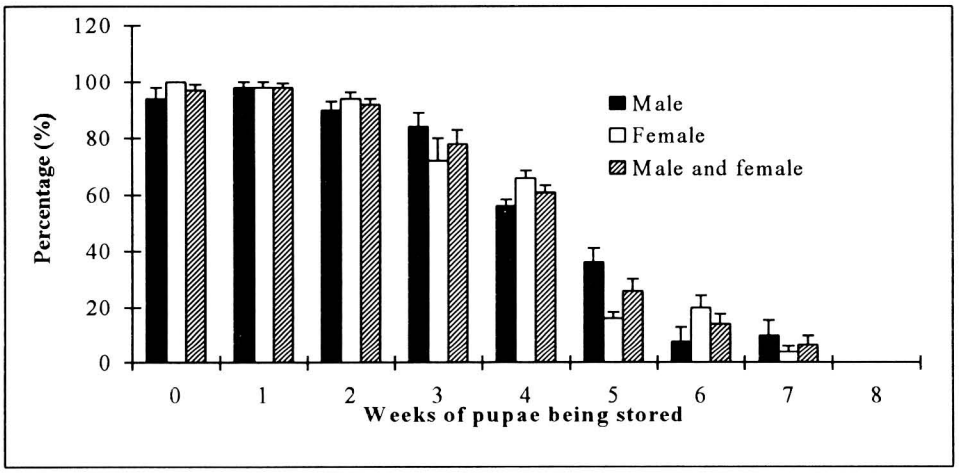

Figure 1. Relationship between cold storage of pupae and adult emergence of *Choristoneura occidentalis*. Vertical bars represent standard errors of mean adult emergence percentage, n (replicate) = 5. Where there are no vertical bars, the emergence from each replicate was the same.

Adult females had significantly longer (F = 14.0; df = 1,4; P = 0.02) lifespans than males, and differences in longevity between the two sexes increased with the length of storage (F = 3.35; df = 7,28; P = 0.03). Longevity of both male and female adults was significantly reduced by cold storage of the pupae (F = 13.1; df = 7,28; P = 0.0001) (Fig. 2).

Effects of Cold Storage on Adult Fecundity. The mean number of eggs laid per female was significantly reduced by cold storage of the pupae (F = 42.0; df = 4,76; P = 0.0001) (Fig. 3 A). The differences in mean numbers of eggs per female between 0 and 1 week cold storage of pupae were not significant (P > 0.05), but egg production was significantly reduced when pupae were stored for longer than 1 week (P < 0.05).

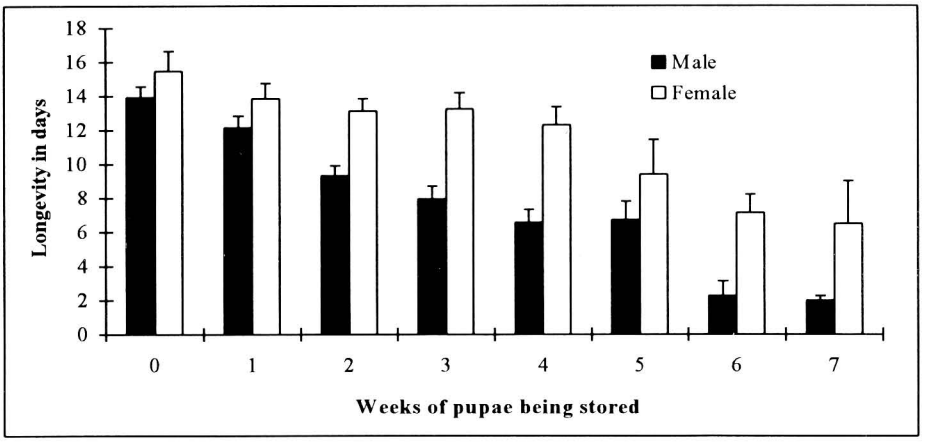

Figure 2. Relationship between cold storage and adult longevity of *Choristoneura occidentalis*. Vertical bars represent standard errors of mean longevity, *n* (replicate) = up to 20, depending on the number of adults emerged in each group.

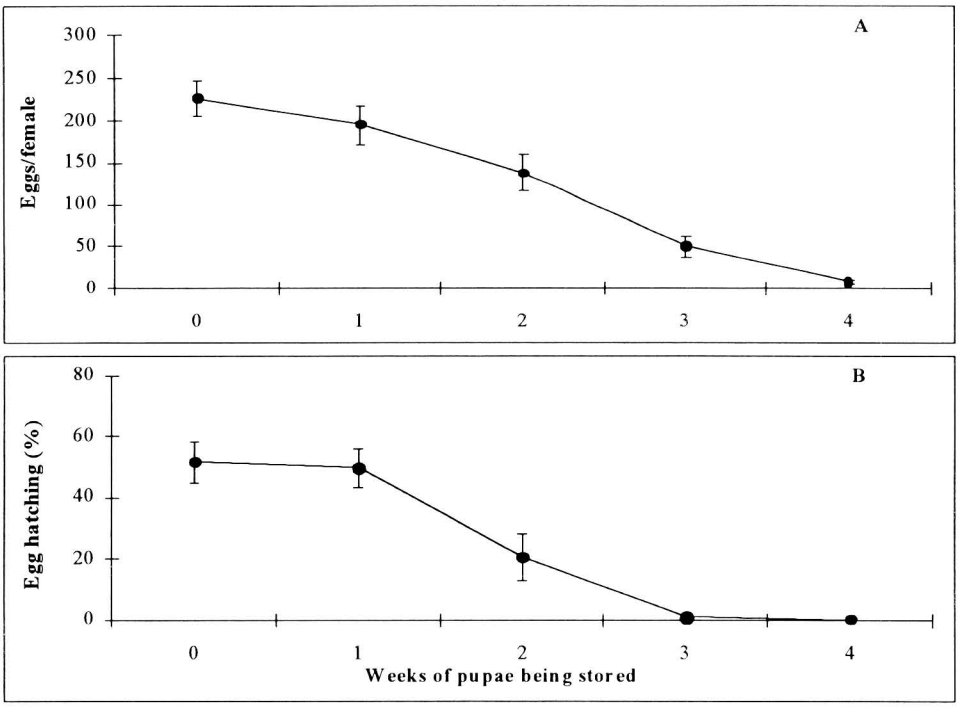

Figure 3. Relationship between cold storage of pupae and the number of eggs laid per female of *Choristoneura occidentalis* (A), and egg viability (B). Vertical bars represent standard errors of mean, *n* (replicate) = up to 20, depending on the number of females that laid eggs in each group. Where there are no vertical bars, mean value from each replicate was the same.

Egg hatching was adversely affected by cold storage of the pupae ($F = 15.4$; df = 3,48; $P = 0.0001$) (Fig. 3 B). Although percentage of egg hatching after 1 week cold storage of the pupae was lower than that for 0 week storage, the difference was not significant ($P > 0.05$). Egg viability was dramatically ($P < 0.05$) reduced after 2 weeks cold storage of the pupae. When pupae were stored for 3 weeks or longer, almost no eggs hatched.

DISCUSSION

Egg hatching rate in this study was only about 50%, for the control pupae that had not received cold storage treatment. The long period of laboratory rearing (> 100 generations) might have caused a deterioration in percentage of egg hatching. Lyon *et al.* (1972) noticed that egg viability of non-diapausing *C. occidentalis* dropped from 88% in the 6th generation to 59% in the 17th generation.

Differences in longevity between adult males and females increased after pupae were subjected to cold storage. The longer the pupae were stored, the greater the differences became. These results indicated that female pupae may have been more tolerant to cold storage than males. We do not know what constituents of female pupae are responsible for the cold tolerance.

The results clearly indicated that cold storage of *C. occidentalis* pupae adversely affected the proportion of adults emerging, adult longevity, mean number of eggs laid per female, and egg viability. After 1 week cold storage of the pupae, all parameters measured were not significantly different from those for 0 week storage. Cold storage of pupae for 2 weeks did not significantly affect adult emergence, but did reduce adult longevity, mean number of eggs laid per female, and egg hatching rate. We conclude that non-diapausing *C. occidentalis* pupae may be stored in darkness at 2.0 ± 0.5 ^{0}C and 100% R.H. for 1 week without deterioration in adult quality.

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