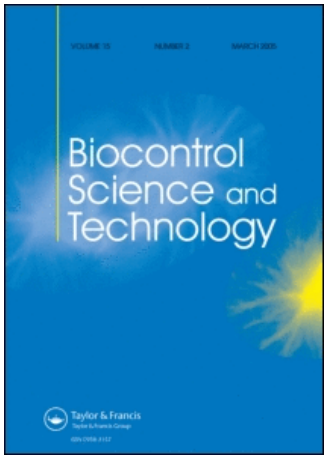

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Cold Storage of *Coleomegilla maculata* larvae

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Short-term storage of second and third instars of the coccinellid Coleomegilla maculata lengi *Timberlake can be achieved efficiently through utilization of temperatures below its thermal threshold of development. Survival, voracity and preimaginal development time were evaluated for larvae kept at 4 and 8°C for up to 5 weeks. Survival was close to 100% for the first two weeks of storage, but decreased drastically afterward and was 0% after 5 weeks. Storage at low temperatures did not diminish the voracity of larvae after storage. Development completely stopped during cold storage but resumed without significant effect after larvae were returned to 24°C. This study indicates for the first time that cold storage of* C. maculata *second and third instars is possible for a period of up to 2 weeks and provides a technique that could benefit biological control programs by increasing availability of beneficial insects.*

Keywords: *mass-rearing, Coccinellidae, larvae, low temperature, storage, predation*

INTRODUCTION

Development of storage techniques can be a useful way to overcome the problem of availability of beneficial insects in biological control programs by reducing rearing costs and increasing ability to meet peak demands from suppliers and users. The utilization of cold temperature is the most common storage technique (Jalali & Singh, 1992; Tauber *et al*., 1993).

Response to temperature varies from one insect to the other. Species that are adapted to low temperatures have a better fitness in a lower range of temperatures (Sehnal, 1991). A large number of insects from temperate regions survive cold temperatures by diapausing. However some species exposed to temperatures between 0 and 10°C will enter a lethargic state that could be reversible if exposure is of short duration (Lee, 1991).

Most of the studies on cold storage considered diapausing stages. The diapausing adult of *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae) was stored with success at 5°C for 31 weeks (Tauber *et al*., 1993). Hämäläinen (1977) succeeded in keeping diapausing adults of the coccinellid *Adalia bipunctata* L. at 6°C for 26 weeks. Jean *et al*. (1990) were able to keep the diapausing adult of *Coleomegilla maculata lengi* Timberlake at −0.5°C for 28 weeks.

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Coleomegilla maculata is a polyphagous coccinellid well distributed in Eastern North America. It can be reared successfully on a liver diet (Gagné *et al*., in prep.) or even on maize pollen for several generations (Hodek *et al*., 1978). Lower thermal thresholds of development for second and third instars are 13 and 11.5ºC, respectively (Gagné *et al*., in prep.). Larvae and adults are voracious predators of aphids (Coderre & Tourneur, 1988), Colorado potato beetle (Giroux *et al*., 1995) and Lepidoptera eggs and young larvae (Andow & Risch, 1985).

Because adult coccinellids tend to leave the field shortly after introduction (Hämäläinen, 1977), less mobile stages were considered in biological control programs. Eggs were evaluated because they are easy to manipulate and can be sprayed in the field (Shands & Simpson, 1972). Their utilization is, however, compromised because a large proportion of eggs can be infertile (Brown, 1972) and because there is a high rate of mortality due to cannibalism (Elgar & Crespi, 1992), intraguild predation (Lucas *et al*., 1998) or environmental factors. They were also found to be difficult to store at low temperatures. Eggs of the coccinellid *Eriopis connexa* Mulsant cannot be stored at 4ºC for more than 24 h (Miller, 1995). Hämäläinen and Markkula (1977) found that *C. septempunctata* and *A. bipunctata* eggs couldn't be kept at 10ºC for more than one and two weeks, respectively.

Despite the fact that manipulation of coccinellid larvae is time consuming, large larvae should be considered in biological control programs because their action against pests is immediate; they are more resistant to adverse climatic conditions than eggs or young larvae (Ferran, 1983); after introduction, they stay longer in the field than adults (Giroux, 1996); and they are generally as voracious as adults (Giroux *et al*., 1995). In order to take advantage of the complete third and fourth instars impact on prey, late second instars should be provided to suppliers. Only a few studies evaluated cold storage of non-diapausing insect larvae. Ferran (1983) concluded that it is not worthwhile to keep *Semiadalia undecimnotata Schn*. third instars at 5ºC or 10ºC for more than 3 days, mortality rate increasing drastically after this period.

This study evaluates the effect of cold storage on second and third instars of *C. maculata*. Survival rate and voracity of larvae after storage were considered in order to select an optimal combination of temperature and duration of storage.

MATERIALS AND METHODS

Insects

Coleomegilla maculata used were the 13th generation of a mass-reared population originally collected at overwintering sites near St-Hyacinthe, Québec (72º56′W, 45º39′N). They were maintained in the laboratory at a constant temperature of 24ºC, a 16:8 (L:D) photoperiod, 60–75% relative humidity (RH) and fed an artificial diet of beef liver and eggs of *Ephestia kuhniella* Zeller (Lepidoptera: Pyralidae). The potato aphid *Macrosiphum euphorbiae* Thomas, used in voracity tests, was maintained on potato plants (Kennebec variety) under the same conditions.

Larval Survival

This experiment was conducted with second and third instars (0–24 h). Previous stages were maintained at a photoperiod of 16:8 (L:D), 24ºC, 60–70% RH and fed an artificial diet of beef liver and eggs of *E. kuhniella*. Because no cannibalism was noted at the temperatures tested, 40 replicates of 10 individuals of each instar were kept in Petri dishes (9 × 50 mm) covered by organdy net to avoid water condensation. Soaked cotton and a small piece of cardboard were placed in each unit to assure humidity and refuge. Larvae were put at 4 or 8ºC at 60–70% RH, under complete darkness and without food.

Each week for 5 weeks, 40 second and 40 third instars were removed from storage and isolated in Petri dishes (9 × 50 mm). Larvae were maintained at a photoperiod of 16:8

(L:D), 24°C, 60–70% RH and fed an artificial diet of beef liver and eggs of *E. kuhniella* for 24 h. Survival was noted after this thermal readaptation period.

Voracity

Predation after storage was measured using three-leaf potato stem sections placed in a hole made in 2 l plastic containers. These were placed in a second container with 200 ml of water to prevent the potato leaflets from drying during the experiment. The system was closed with organdy held by a perforated lid on the top container.

Twenty larvae from each temperature-storage duration treatment were individually placed on a previously infested leaf with either 20 nymphs of *M. euphorbiae* for second instar or 30 nymphs for third instar treatments. Twenty second and third instars that were kept at 24°C were used as controls. Experiments were conducted under a photoperiod of 16:8 (L:D), at 24°C and 60–70% RH. Number of prey missing after 24 h was calculated. Aphid mortality data were corrected by subtracting the mean mortality observed in the controls (same system without predator).

Preimaginal Development

Only 1 and 2 weeks of storage treatments were considered due to high mortality observed after this period. Time of development of each instar was observed daily and only individuals that successfully completed molting were considered. Coccinellids were maintained under a photoperiod of 16:8 (L:D), at 24°C and 60–70% RH until imaginal stage was reached. Twenty second and third instars that were reared at a constant temperature of 24°C were used as controls.

Statistical Analyses

Survival was compared between weeks with a χ^2 analysis and between treatments within the same week with G-test (Abacus Concepts, 1992). Treatments were compared to controls with the Dunnett's Method (Sall & Lehman, 1996). One- or two-factor ANOVAs, followed when needed by Fisher's Protected LSD test, were used to compare treatments for voracity (Abacus Concepts, 1989).

RESULTS

Larval Survival

At both temperatures, duration of storage significantly affected survival of second (L2) and third (L3) instars (L2 at 4°C: $\chi^2 = 113.6$, df $= 4$, $P < 0.0001$; L2 at 8°C: $\chi^2 = 90.6$, df $= 4$, $P < 0.0001$; L3 at 4°C: $\chi^2 = 138{,}5$, df $= 4$, $P < 0.0001$; L3 at 8°C: $\chi^2 = 118.5$, df $= 4$, $P < 0.0001$). No significant differences were found between treatments after 1 week (Figure 1). However, after 2 weeks, survival of L2 at 4°C, was significantly lower than in the three other treatments (Figure 1). After 3 weeks, survival decreased by half. Survival of second and third instars was significantly higher at 8°C than at 4°C. No differences were observed, however, between stages at the same temperature. Survival was close to 0% for all treatments after 5 weeks.

Voracity

Storage of second instar larvae at 8°C for as long as 4 weeks did not reduce their voracity compared to larvae that were kept at 24°C (Dunnett's method, $P > 0.05$) (Figure 2). Second instars stored at 8°C for 1 week even significantly increased the number of aphids eaten after storage (Dunnett's method, $P < 0.05$). However, longer-term storage (3 or 4 weeks) significantly reduced voracity of surviving larvae (Figure 2). Voracity of second instars significantly decreased after storage at 4°C (Dunnett's method, $P < 0.05$). Voracity of all L3 treatments were significantly lower than controls after 3 and 4 weeks. Voracity of L2 kept at 4°C was significantly lower than the voracity of L2 kept at 8°C (ANOVA $F = 14.823$,

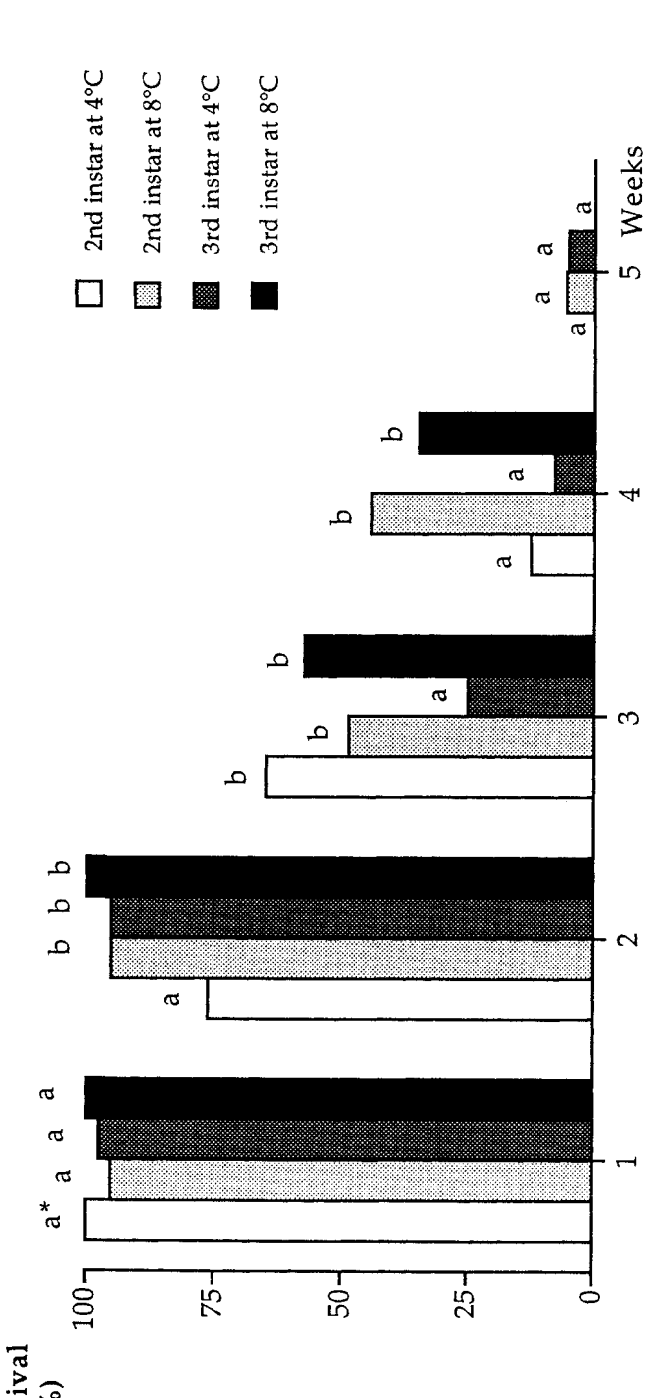

FIGURE 1. Survival of second and third instar larvae of *C. maculata lengi* at low temperatures. Different letters indicate a significant difference ($P < 0.05$) between treatments within the same week.

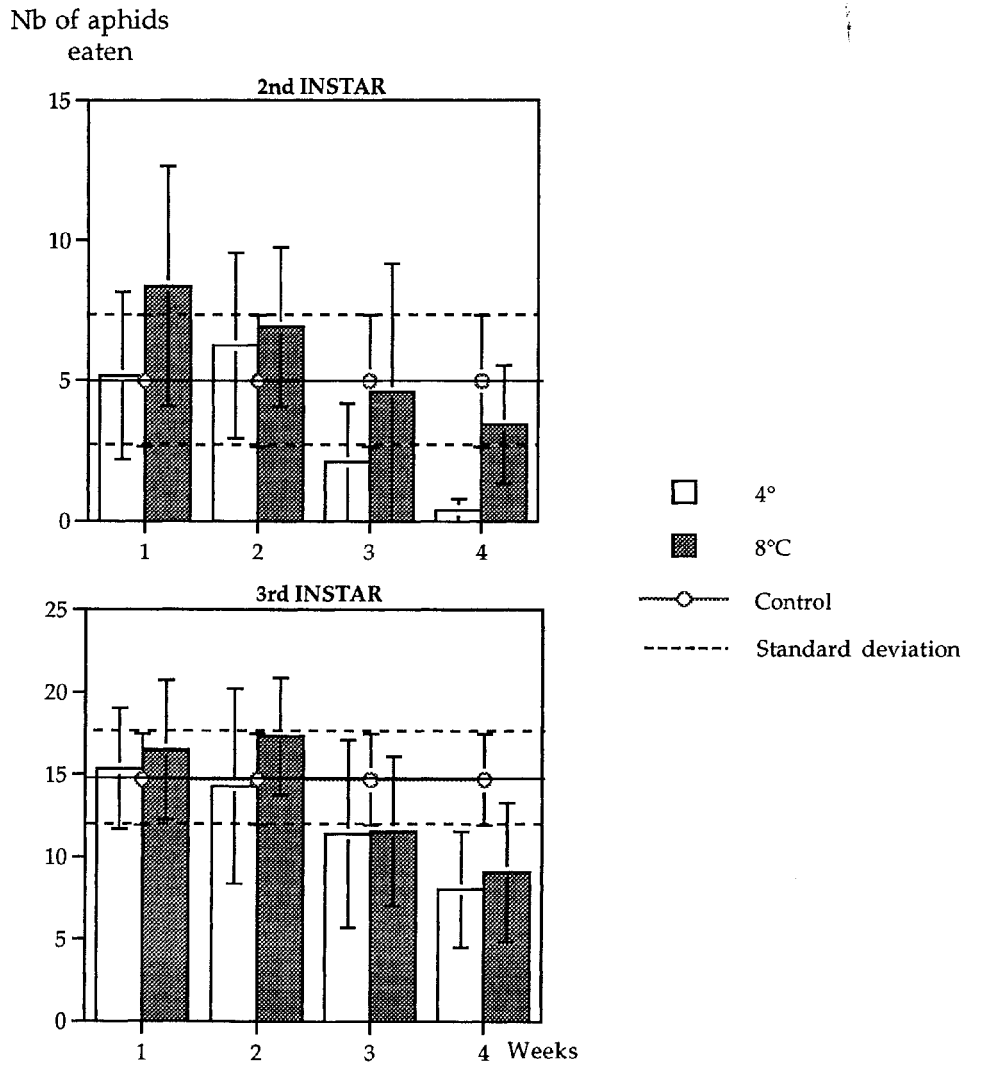

FIGURE 2. Voracity of second and third instar larvae of *C. maculata lengi* after storage at low temperatures.

df = 1.108, $P = 0.0002$) (Figure 2). The same pattern was found for third instar larvae but was not significant (ANOVA $F = 1.825$, df = 1.118, $P = 0.1794$).

Preimaginal Development

Time required to complete the second or third instar after storage at 4°C or 8°C was significantly higher than for larvae without storage (Dunnett's method, $P < 0.05$) (Figure 3). Developmental time of fourth instar larvae was not affected negatively by storage (Dunnett's method, $P < 0.05$), (Figure 4). Nymph developmental time was, however, longer when L2 or L3 larvae were kept at 4°C for 1 week (Dunnett's method, $P > 0.05$) (Figure 4).

DISCUSSION

Our results show for the first time that it is possible to store second and third instars of *C. maculata* at low temperatures for up to 2 weeks without significant mortality. Moreover,

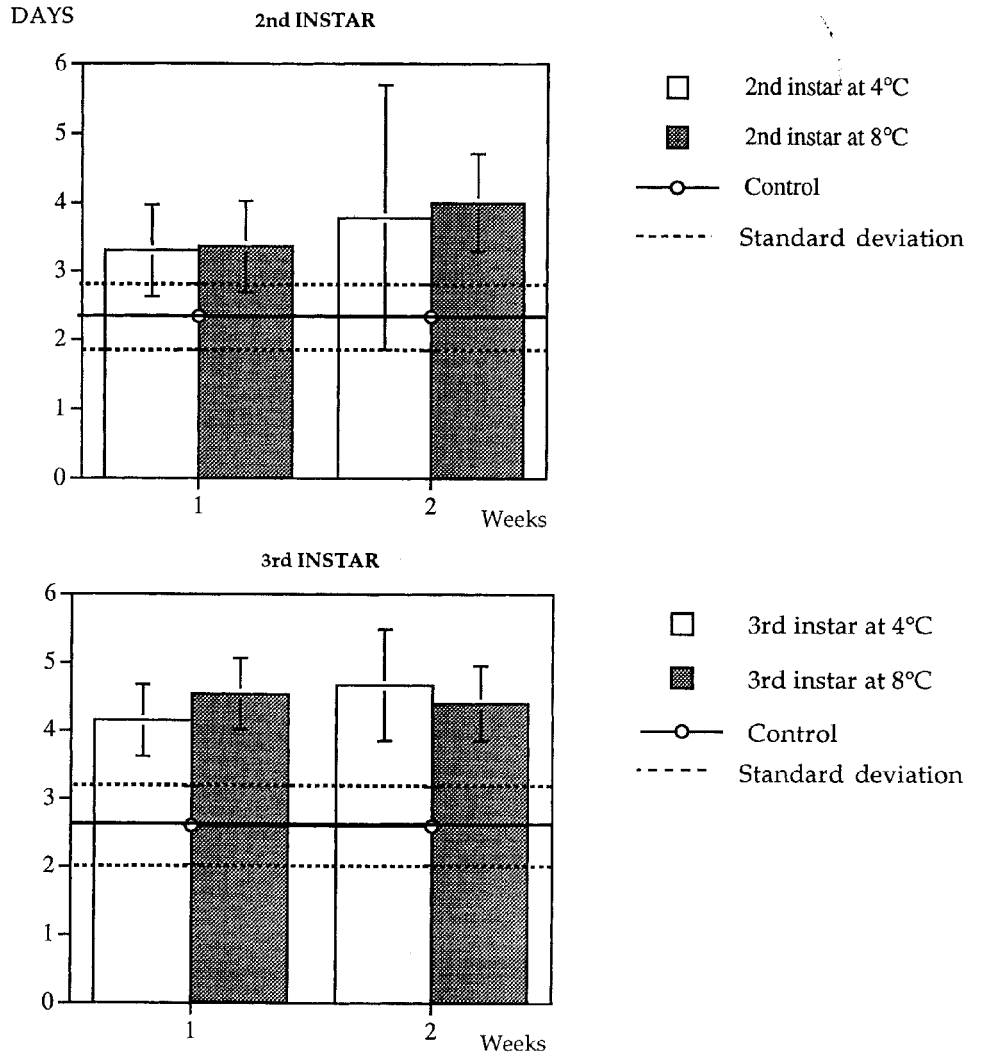

FIGURE 3. Preimaginal development time of second and third instar of *C. maculata lengi* larvae after storage at low temperatures.

voracity significantly increased after storage. Compared to 4°C, storage at 8°C resulted in better survival, higher voracity and faster rate of development when larvae were returned to 24°C. Our results corroborate those of Hämäläinen and Markkula (1977) who found that best storage temperatures for *A. bipunctata* and *C. septempunctata* eggs were the highest of the three temperatures tested (3, 6.5 and 10°C). Jalali and Singh (1992) also found that the highest of three temperatures tested (2, 5 and 10°C) gave the best results for the storage of four *Trichogramma* species for a short period, as Iacob and Iacob (1970) who found that storage of *Trichogramma evanescens* Westwood was optimal between 9 and 12°C. These results suggest that storage at temperatures over 8°C, but under the thermal threshold of development (12.2°C), could result in a better survival rate than we obtained in this study. This should be tested in future studies.

Hämäläinen and Markkula (1977) noted that developmental time of coccinellid eggs kept

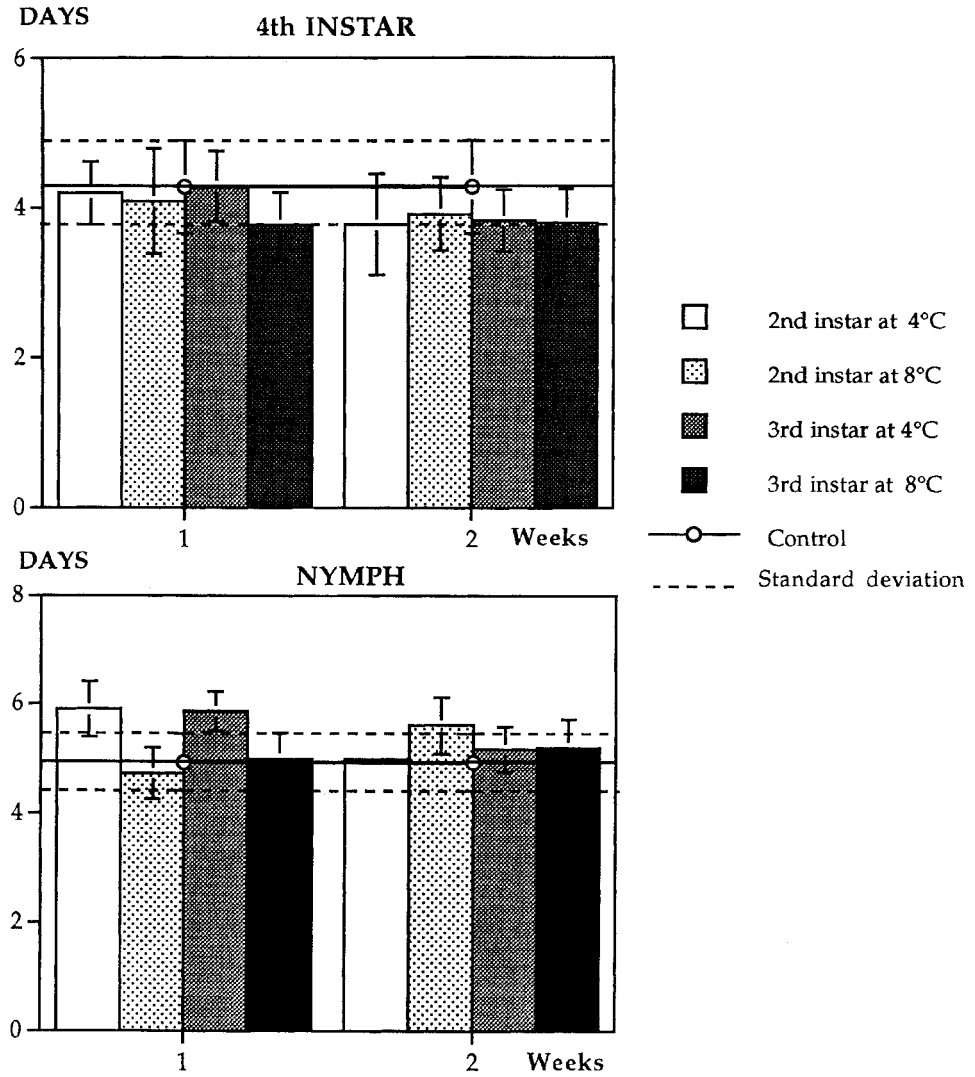

FIGURE 4. Preimaginal development time of *C. maculata lengi* larvae after storage at low temperatures.

at 10°C increased with duration of storage. Ferran (1983) indicated that third instars of *S. undecimpunctata* molted when kept at 10°C. Neither of those situations occurred in our study when second and third instars *C. maculata* were kept at 4 or 8°C. Contrary to these studies, where storage temperature tested allowed slow development (Sehnal, 1991), the temperatures used in our study were below the thermal threshold of development for *C. maculata* (Gagné *et al.*, in prep.) and completely stopped development.

Our study shows that larval development can resume after cold storage, as suggested by Sehnal (1991), Danks (1987) and Mansingh (1971). Sehnal (1991) suggested that survival and development of insects stored at low temperatures could be affected if storage lasts for a long time, but no significant effect should be found for a short period. Our results and those of Jessup and Baheer (1990) on *Dacus tryoni* (Froggatt) corroborate this. According to our results, 2 weeks should be considered as a short period of storage because survival was generally close to 100% for both temperatures tested (Figure 1).

Larvae kept at low temperatures slow their metabolism (Chapman, 1982) and can survive on their fat bodies for a longer time. Therefore, larvae starved for 2 weeks at low temperature could be similar to larvae starved for a shorter period of time under normal conditions. This could explain similar or higher voracity (L2 at 8ºC for 1 week) of stored larvae than control larvae that we obtained (Figure 2).

Storage at temperatures below thermal threshold of development for more than 3 weeks was lethal for more than 40% of *C. maculata* larvae (Figure 1). Prolonged exposure to low temperatures could have caused cellular damage (Chen *et al*., 1987), metabolism perturbations (Chapman, 1982; Knight *et al*., 1986) or accumulation of toxins (Chapman, 1982). Specific interference with neurohormones responsible for insect development could also be lethal, particularly when insects are exposed to suboptimal temperatures for a long time (Pullin & Bale, 1988).

Voracity is also affected after three weeks of storage (Figure 2). The loss of mobility that we observed in larvae is the main factor affecting their predation efficacy after storage. This sublethal effect could be related to cellular damage (Chen *et al*., 1987) or metabolism perturbation (Chapman, 1982) that can affect coordination and movement ability.

For many insect species, acclimatization before storage at low temperatures leads to high survival by avoiding thermal shock (Denlinger, 1991). However, in our experiment no thermal shock was noted since 100% of *C. maculata* larvae survived the first week of storage. It is therefore doubtful that acclimatization could increase survival for a longer storage. However, this should be tested.

Cold storage of *C. maculata* second and third instars at 8ºC for a period up to 2 weeks could benefit biological control programs by providing flexibility in mass-production and short-term storage technique for suppliers and users.

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