

COLD-TOLERANCE IN THE TENT CATERPILLAR *MALACOSOMA AMERICANUM* AND THE EFFECTS OF HORMONES

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## ABSTRACT

The chill-coma temperature and the supercooling point of the third instar larvae of *Malacosoma americanum* were  $7.0^{\circ}\text{C} \pm 0.4^{\circ}\text{C}$  and  $-14.5^{\circ}\text{C} \pm 1.5^{\circ}\text{C}$  respectively. Treatment of these larvae with 1 or  $4\mu\text{l}$  of ecdysterone or farnesyl methyl ether had no significant effect on these cold-tolerance values, though there is some indication that the growth hormone may reduce cold-tolerance by raising supercooling point.

The chilled larvae could regain locomotory activity within 8 minutes of being transferred to  $30^{\circ}\text{C}$  from 3 weeks of chilling at  $5^{\circ}\text{C}$ . The hormones had no apparent effect in the acceleration of recovery.

## INTRODUCTION

**H**ORMONAL regulation of various life processes in insects has been demonstrated in many species. Diapause or arrest of growth and development is also attributed to the lack of brain hormone, ecdysone or juvenile hormone<sup>1</sup>. However, the control of other forms of dormancy such as quiescence and oligopause is still not clear<sup>2</sup>.

Topical application of farnesyl methyl ether (FME) was found to increase cold-tolerance in the larvae of the wax moth *Galleria mellonella* L.<sup>3</sup>. The time required for recovery from quiescence and the overall mortality, after 1 to 3 weeks of chilling at  $4^{\circ}\text{C}$ , were significantly reduced. The results were explained on the basis of the effects of the chemical on the general metabolism in lepidopterous larvae.

In the present study, the possible effects of ecdysone and FME on the chill-coma temperature, as defined by Mutchmor and Richards<sup>4</sup>, supercooling point and temperature acclimation were investigated on the larvae of the eastern tent caterpillar *Malacosoma americanum* (Fab.) (Lasiocampidae, Lepidoptera).

The biology of *M. americanum* is well suited for the present study. The species is found throughout southern belt of Eastern Canada, and almost all over United States, east of Rocky Mountains<sup>5</sup>. The overwintered fully mature embryos hatch in early May and complete larval development within 5–6 weeks. The adult females lay eggs in early July and the embryonic development takes about 3–4 weeks.

## MATERIALS AND METHODS

The tent caterpillars for this study were collected from wild cherry trees around Kingston (Ontario) and reared in the laboratory at  $30^{\circ}\text{C}$  as described by Mansingh<sup>5</sup>.

Third instar larvae were either injected with 1 or  $4\mu\text{l}$  of ecdysterone (supplied by Rohoto Pharmaceuticals, Osaka, Japan) as 10% ethanol solution

or topically treated with 1 or  $4\mu\text{l}$  of FME (supplied by Flunkaag, Buchs, Switzerland). The control larvae received ethanol injection or acetone application. One day after the hormonal treatment, the larvae were transferred to  $5^{\circ}\text{C}$  for 3 weeks, or maintained at  $30^{\circ}\text{C}$ . At the end of the chilling period, the chilled larvae were brought back to  $30^{\circ}\text{C}$  and the time for their recovery from cold-torpor was recorded according to Mansingh<sup>3</sup>. The growth rate during the chilling was determined by weighing the larvae before and after cold-exposure.

The chill-coma temperature was determined by exposing the larvae from  $30^{\circ}\text{C}$  to a series of low temperatures for 1 hour. The temperature at which they became inactive was taken as the chill-coma temperature. Supercooling points were measured with 32-gauge copper-constantan thermocouple while the rate of cooling was  $2^{\circ}\text{C}/\text{minute}$ <sup>5</sup>.

## RESULTS AND DISCUSSION

The amounts of ecdysone or FME used in the present investigation had no apparent effect on the larval development at  $30^{\circ}\text{C}$  or  $5^{\circ}\text{C}$ . Cold exposure had induced torpor within half an hour, and no feeding activity was noticed during the 3 weeks of chilling period. Both the treated and controlled larvae lost about 8.25% of their original weight of 0.26 g, during the cold exposure.

Hormonal treatment had no effect on the chill-coma temperature of the larvae which remained unchanged at around  $7.0^{\circ}$  (Table I). Similarly, the supercooling ability of the larvae was also not significantly altered by the hormones, though higher dose of ecdysone ( $4\mu\text{l}/\text{larva}$ ) appear to reduce cold-tolerance by slightly increasing the supercooling point. This is not surprising because chilling and ecdysone treatment have antagonizing effects on the metabolism. Low temperature treatment reduces metabolism and biosynthetic activities<sup>2,6</sup>; ecdysone, on the other hand, stimulates RNA and protein syntheses<sup>7</sup>. This aspect merits further investigation.

TABLE I  
Effect of hormones on the cold-tolerance of *M. americanum*

Treatment	Chill-coma temperature (°C)	Supercooling points (°C)
Control	7.0±0.4	-14.5±1.5
Ecdysone — 1 µl	6.8±0.3	-15.2±1.1
Ecdysone — 4 µl	6.5±0.4	-15.5±0.5
FME 1 µl	7.5±0.2	-13.8±1.0
FME 4 µl	7.8±0.5	-12.8±0.8

20-30 larvae/experiment.

Table II shows that the locomotory activity in the chilled larvae was regained within 10 minutes ( $8 \pm 1.25$  minutes) of their return to 30°C. FME or ecdysone had no apparent effect on the recovery time.

TABLE II  
Effect of hormones on the recovery of locomotory activity in *M. americanum* larvae exposed to 25°C after 3 weeks of chilling at 5°C.

Treatment	% larvae recovered			
	4	6	8	10 (min)
Control	25	45	84	100
Ecdysone — 1 µl	20	40	90	100
Ecdysone — 4 µl	35	50	100	100
FME 1 µl	28	45	90	100
FME 4 µl	34	52	88	100

The anomaly in the role of FME in the induction of cold-tolerance in *M. americanum* and *G. mellonella*<sup>3</sup> may be due to the inherent differences in the level of cold-tolerance in the two species. This is evidenced by the ability of tent caterpillar larvae to recover from cold-torpor within 10 minutes, even when the chilling induced irreversible and fatal injury in over 60% of the larvae<sup>5</sup>. The wax moth

larvae, on the other hand, required about 15 to 40 minutes to recover, depending upon the duration of chilling.

Intraspecific differences in the two species is further indicated by significant differences, in their chill-coma temperatures. The low chill-coma temperature of the tent caterpillar is almost equal to that of *Musca domestica* or *Sarcophaga bullata*; for the larvae of *G. mellonella*, it is 8.8°C<sup>4</sup>. Mutchmor<sup>6</sup> has discussed the positive correlation between the chill-coma temperature, muscle apyrase activity, ATP-ase activity and cold-tolerance in a number of species; the lower the chill-coma temperature, the more cold-tolerant is the species. Thus the inherently high cold-tolerance in the tent caterpillar can apparently not be further increased by the low doses of FME.

Increased cold-tolerance and the ability to recover quick from cold-torpor have adaptive significance for the tent caterpillar. It is not unusual for the insect to experience temperatures which fluctuate between 0 and 25°C; the wax moth larvae rarely face such adversity. Consequently, *M. americanum* larvae have evolved the ability to adapt quickly to the overnight low temperatures, prolonged spells of cold or high day temperatures.

#### ACKNOWLEDGEMENTS

Prof. B. N. Smallman, Department of Biology, Queen's University, Kingston, Ontario, Canada, had kindly provided the laboratory facilities, and the U.W.I., the travel grant.

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