

Cold hardiness of black carpet beetle larvae

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In North America, the black carpet beetle, *Attagenus unicolor* (Brahm) (Coleoptera: Dermestidae), is a common household insect pest that feeds on many animal-origin and grain products. It overwinters as a larva, and the entire life cycle generally takes about 1 year. No information exists on its overwintering biology or cold hardiness (Fields 1992). In this experiment, the supercooling point (SCP) and survival rate of initially overwintering *A. unicolor* larvae collected in the nests of tree swallows, *Tachycineta bicolor* (Vieillot) (Aves: Hirundinidae), were measured. Changes in SCP and cold hardiness were determined for larvae held at 15 and -15°C . The cold hardiness of *A. unicolor* larvae collected from a feed mill in British Columbia was also measured.

Fifty tree swallow nests, which yielded 220 *A. unicolor* larvae, were collected on 16 February 2000 at Delta Marsh, Manitoba ($50^{\circ}10'N$, $98^{\circ}20'W$), at an air temperature of approximately -20°C . Nests were stored in the dark at $-15 \pm 1.5^{\circ}\text{C}$ in sealed plastic bags. Larvae were separated from nests at room temperature (about 15 min at 20°C for each nest) and returned to -15°C . The SCP of 20 larvae was taken 8 h later. The remaining larvae were randomly divided into two equal groups. One group was placed, together with debris from the bird nests as food, at $15 \pm 1.5^{\circ}\text{C}$ and 50% RH for 1 week, and the other was kept at -15°C and 50% RH for 1 week. The initial mortality of the overwintering larvae was recorded in the former group at 15°C . Live larvae were plump and light brown, whereas dead larvae were dry and dark brown. Ten larvae that had been held at -15°C for 1 week were moved to 15°C with food for another week, to determine the change in their SCP. Fifty live larvae were selected from each group, from among which 10 were used to measure SCPs and forty were placed at $-25 \pm 1.5^{\circ}\text{C}$ for 1, 3, 5, or 7 d. Ten larvae from each group were held at $25 \pm 1^{\circ}\text{C}$, 70 \pm 5% RH, and a 16L:8D photoperiod with food for 1 week, and their survival noted.

Fifty *A. unicolor* larvae were collected from a feed mill in Abbotsford, British Columbia ($49^{\circ}03'N$, $122^{\circ}17'W$), on 27 June 2000. SCPs of 10 larvae were measured. Groups of 10 larvae, together with 1 g of ground dog food, were placed separately at -25 ± 1.5 , -15 ± 1.5 , -10 ± 1.5 , or $-5 \pm 1.5^{\circ}\text{C}$ for 24 h. Mortality was recorded after these larvae were cultured individually at $25 \pm 1^{\circ}\text{C}$, 70 \pm 5% RH, and a 16L:8D photoperiod for 1 week.

SCPs were measured using a data logger (CR7, Campbell Scientific Ltd). An insulated test chamber (40-cm diameter) was placed in a freezer at $-45 \pm 3^{\circ}\text{C}$. The cooling rate was approximately $1^{\circ}\text{C}/\text{min}$. Dry ice was used to maintain a constant cooling rate at lower temperatures. Larvae were placed beside a thermocouple at the bottom of

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TABLE 1. Mortality of field-collected *Attagenus unicolor* larvae in Manitoba in winter held at -15 or 15°C for 1 week and exposed to -25°C for different lengths of time.

Days at -25°C	Pre-exposure temperature*			
	-15°C		15°C	
	<i>n</i>	Mortality (%)	<i>n</i>	Mortality (%)
1	10	0a	10	20a
3	10	30b	10	90b
5	10	40b	10	100b
7	10	40b	10	100b

* Pre-exposure temperature had significant effect on mortality (SAS GENMOD, $P < 0.001$). For a given temperature, values followed by a different letter are different (SAS GENMOD, $P < 0.05$).

TABLE 2. Supercooling points of *Attagenus unicolor* larvae collected from nests of *Tachycineta bicolor* at Delta Marsh, Manitoba, in winter and subjected to different temperature treatments, and of those collected from a feed mill in British Columbia in summer.

Treatment	<i>n</i>	Supercooling point ($^{\circ}\text{C}$)	
		Mean \pm SE*	Range
Initial in winter	20	$-35.6 \pm 0.9a$	-30.3 to -39.0
1 week at -15°C	10	$-35.8 \pm 0.8a$	-33.1 to -39.3
1 week at 15°C	10	$-25.2 \pm 2.0b$	-19.7 to -33.0
1 week at -15°C then 1 week at 15°C	10	$-24.9 \pm 0.4b$	-23.3 to -25.7
Summer	10	$-15.9 \pm 1.2c$	-9.8 to -21.2

* Means followed by different letters are different (Student–Newman–Keuls method, $P < 0.05$).

an Eppendorf pipette tip. The SCP was taken as the lowest temperature before the sharp rise in temperature that was due to the heat of crystallization.

SAS GENMOD procedure (SAS Institute Inc 1997) was used to compare the difference in mortality between the pre-exposure temperatures and among exposure durations. The Student–Newman–Keuls method was adopted for testing the differences between supercooling points under different treatments.

Attagenus unicolor larvae ($n = 100$) taken directly from the overwintering site and held at 15°C and 50% RH for 1 week had 33% mortality. When exposed to -25°C , larvae that had been held at 15°C were found to be less cold hardy than those maintained at -15°C ($\chi^2_1 = 31.53$, $P < 0.001$; Table 1). Mortality at 25°C at each pre-exposure temperature increased with the duration of exposure (for those held at -15°C , $\chi^2_3 = 7.92$, $P < 0.001$; for those held at 15°C , $\chi^2_3 = 21.98$, $P < 0.001$; Table 1). The mortality of larvae from the feed mill held for 24 h at -25 , -15 , -10 , or -5°C was 100, 70, 10, or 0%, respectively. The mortalities of larvae were significantly different among temperatures ($\chi^2_3 = 36.33$, $P < 0.05$), except for those in -25°C compared with those in -15°C ($\chi^2_1 = 1.44$, $P < 0.23$).

Larvae held at 15°C for 1 week froze at a higher temperature than larvae taken directly from their overwintering site or held at -15°C ($F_{1,3} = 25.19$, $P < 0.05$; Table 2). There was no significant difference in SCP between larvae taken directly from the nests and those held at -15°C for 1 week. There was no difference in SCP between larvae held at 15°C and those moved from -15 to 15°C . The SCP of larvae from the feed mill in the summer was higher than the SCP of all overwintering larvae in all treatments ($F_{4,39} = 60.3$, $P < 0.05$).

The SCP of insects usually changes seasonally (Lee 1991; Danks 1996). *Attagenus unicolor* has a low SCP in the winter that increases in the spring and summer, as in our simulated spring temperature of 15°C for 1 week and in the feed mill in the summer. The SCP of overwintering larvae, -36°C, was close to the minimum temperature in southern Manitoba during the winter of 1999-2000. In addition, *A. unicolor* overwinters in birds' nests, where the extreme low temperature is probably slightly warmer than minimum temperature, because of the insulation afforded by feathers, straw, and sometimes snow. Low temperatures are often used to control insects that infest stored products (Fields 1992). Although the temperatures needed to kill *A. unicolor* larvae are too low to control an infestation in a building during the summer, freezing may be a practical method for controlling this insect in finished products. The cold hardiness of other life stages needs to be measured because it may differ from that of the larvae (Fields 1992).

Despite a SCP that might be lower than the minimum temperature of their habitat, some overwintering *A. unicolor* larvae do not survive until spring. Turnock (1993) reported that the mortality of insects collected in Manitoba increased with length of exposure time, even at a temperature above the SCP. Similar trends were observed here: the longer that *A. unicolor* larvae were exposed, the higher their mortality. Therefore, cold injury occurs in *A. unicolor* larvae at temperatures above their SCP.

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