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COLD-HARDENING OF THE GROUND BEETLE *CARABUS GRANULATUS* L. (*COLEOPTERA, CARABIDAE*)

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Abstract

Cold-hardiness is the ability of insects to survive low temperatures, with or without freezing. Supercooling point (SCP) is the temperature at which spontaneous freezing occurs. The aim of present research was to study seasonal cold adaptation dynamics of the ground beetle *Carabus granulatus* L. (*Coleoptera*, *Carabidae*). Seasonal changes in mean SCP values in *C. Granulatus* were determined as follows: −4.4^{o} C (in January 2007), −5.3^{o} C (in May 2007), −6.3^{o} C (in September 2007) and −5.6^{o} C (in February 2008). The tests showed weak supercooling ability of this species and according to Hawes and Bale (2007) classification *C. granulatus* belongs to freeze-tolerant cryotype.

Key words: *Carabus granulatus*, *Carabidae*, cold-hardiness, seasonal dynamics, supercooling.

Introduction

Insect seasonal adaptation depends on many abiotic factors among which temperature is the most critical factor aside of humidity and light /Thiele, 1977; Danks, 2006/. Cold-hardiness is the essential component of winter survival for most insects in the temperate zone /Denlinger, 1991/. At temperatures below 0^{o} C, most insect species remain unfrozen because they supercool. Cold-hardiness can be measured by a number of indices such as supercooling points (SCP), the temperature at which spontaneous freezing occurs /Danks, 2004/. Cold-hardy insects have been divided into two groups: those tolerant of internal freezing and those susceptible to freezing. Insects that are freezing-tolerant survive ice formation in body tissues. Ice formation usually is confined to extracellular fluids, thus avoiding damage to intracellular components. Protective mechanisms for freezing resistance include elevated solute levels, presence of nucleating agents, and accumulation of cryoprotectants in body fluids. A number of freeze-tolerant insects have supercooling points in the range of −8^{o} C to −10^{o} C, whereas some freeze-susceptible species supercool extensively, to −60^{o} C or below. Insects that supercool are freezing-susceptible since they avoid freezing damage to body tissues only at a temperature above the supercooling point. Below the supercooling point, death results from ice formation in body tissues /Lee, 1989; Danks, 2004/. These relationships are summarized in Figure 1 adapted from Lee (1989) and Ellsbury et al. (1998).

Cold-hardiness is equally important to pests and beneficial organisms. Frequent beneficial species are ground beetles (*Coleoptera*, *Carabidae*). Ground beetles are species rich and abundant in habitats worldwide and due to their predatory polyphagous nutrition, they are potentially important as natural pest control agents for the agricultural landscape /Thiele, 1977; Kromp, 1999; Symondson et al., 2002/. Adult carabid beetles exhibit scarcely any activity in winter. Carabid species that inhabit in cultivated fields spend the winter in soil or migrate into the field boundaries, a part of species migrate into the forest /Thiele, 1977/. The microenvironment of overwintering sites is at least as important in allowing winter survival as are physiological adaptations /Danks, 1991/.

The aim of the present research was to study seasonal cold adaptation dynamics of the ground beetle *Carabus granulatus*.

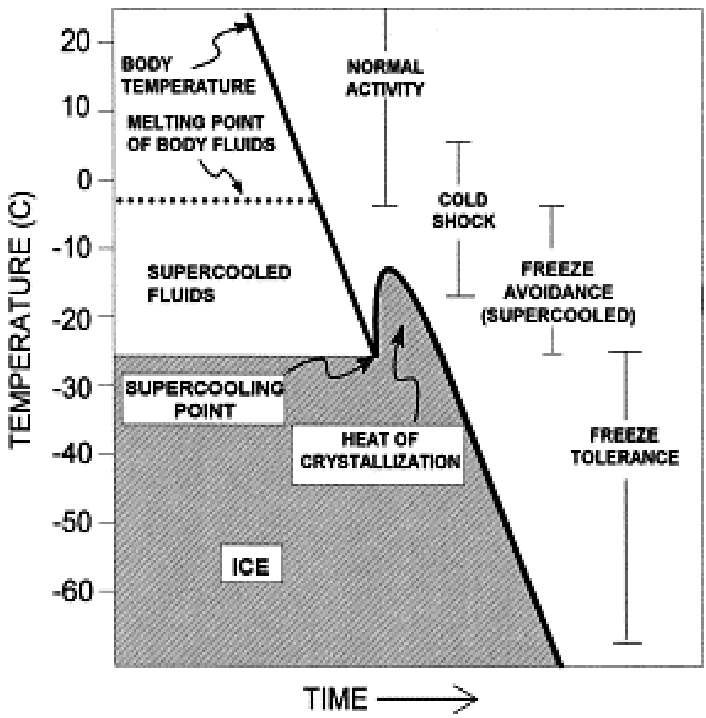

Figure 1. The generalized diagram of insect response to subzero temperature. Insect body temperatures (heavy line) in relation to the melting point, the supercooling point, and the nucleation of ice in body fluids. The bars on the right convey general ranges of insect response to low temperatures, the top of the bar for the range of freeze tolerance and the bottom of the bar for freeze avoidance correspond to the supercooling point value illustrated in the center of the figure /Lee, 1989; Ellsbury et al., 1998/

Materials and Methods

Insect

Carabus granulatus Linnaeus, 1758 is a 16–23 mm large granulate bronze, green or black beetle. It feeds mainly on other insects including agricultural pests. *C. granulatus* inhabits forest and adjoining arable fields, and is also to be found among the 50 most widespread field carabids. *C. granulatus* reproduces in next spring and its larvae live underground /Thiele, 1977/. Beetles hibernate during winter in the forest and can be found on the sheltered side of older and partially decayed tree stumps, adult beetles bury themselves 5–10 cm in the ground beneath the rotten layer /Scherney, 1955/.

Insect collecting

Before experiment adult *C. granulatus* were collected in the field by using dry pitfall traps (May and September 2007), and in wintertime (January 2007, February 2008) beetles could be found in their hibernating sites – tree stumps. Beetles were collected from the Tartu County, Estonia.

Laboratory tests

Supercooling point and low temperature survival were determined using a 0.5^o C min^{-1} cooling rate. The adult insect was positioned so that its integument (thoracic tergit) was in contact with the copper-constantan thermocouple, placed in glass vial, and then transferred to circulator bath (Ministat 125w-2, Huber, −25^o C to +150^o C). The temperature was registered and saved by using data logger (Almemo 2890-9, Ahlborn). Probit analysis by Finney (1971) was used for calculation of 50 and 90% lethal mortality temperatures (LT_{50} and LT_{90}) after 24 hours influence. Lethal mortality temperature measurements were carried out in January 2008.

Statistical comparisons were performed with repeated-measures ANOVA by Tukey test. All means were considered significantly different at the $P<0.05$ level.

Results and Discussion

Mean supercooling points are shown in Figure 2. Seasonal mean SCPs examined in *C. granulatus* were determined as follows: −4.4^o C (in January 2007), −5.3^o C (in May 2007), −6.3^o C (in September 2007) and −5.6^o C (in February 2008). 24 hours exposition in the temperature of −7.4^o C was lethal to the 50% of the beetles and −7.7^o C caused 90% of insects mortality. Our results showed that *C. granulatus* adults had quite high SCPs through year. All insects survived SCPs having no cold injuries even when insects were kept in SCP temperature for 3 days. Thus, the *C. granulatus* is freezing-tolerant species having though a weak supercooling ability /Hawes, Bale, 2007/.

According to the Estonian Meteorological and Hydrological Institute's (EMHI) measurements the mean ground temperatures at insect overwintering time were −1.7^o C (January, 2007) and 0.3^o C (February 2008). These temperatures are quite high and do not cause any harm to *C. granulatus*. Potentially freezing temperatures may occur for short periods. Snow cover protects against cold, because snow is an important insulator against low temperatures, and a cover of snow or vegetation also helps overwintering individuals against lethal temperatures /Danks, 2004/. Even 1.25 cm snow cover raises soil temperature at 5 cm depth and 2.5–5 cm snow cover could have a significant effect on insect overwintering /Mail,1930/. As a result, during cold periods with snow cover insects are protected, but cold periods without snow could be lethal. Mail (1930) measured soil temperature (without snow cover) when the air temperature was −24.2° C and found that at 5 cm depth the temperature was −17.94° C, at 20 cm depth −15.24° C, at 40 cm depth −9.78° C, and at 60 cm depth −5.46° C.

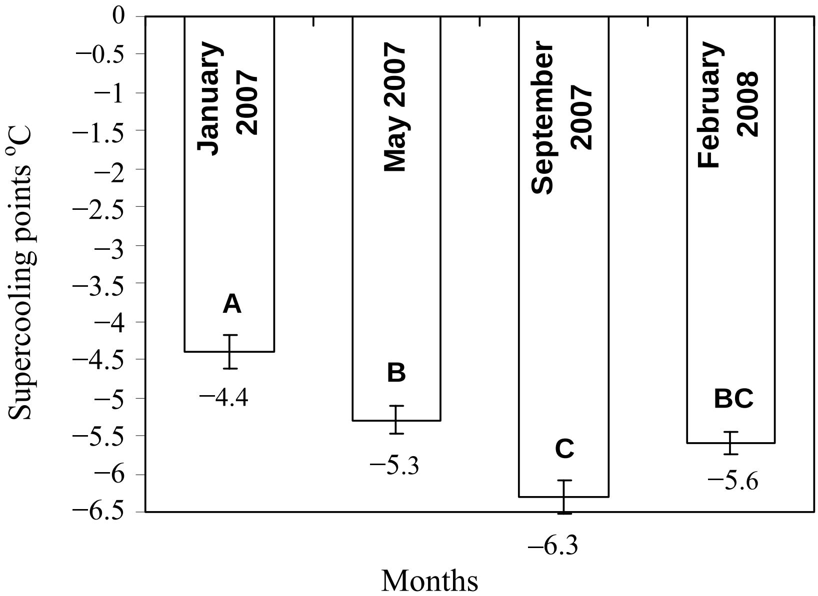

Photo: Jevgenij Chibilev

Figure 2. Cold-hardiness seasonal dynamics of the ground beetle *Carabus granulatus*. Columns with different letters are significantly different (Fisher LSD test $p<0.05$)

The SCP was lowest in September 2007 (−6.3° C) and highest in January 2007 (−4.4° C) ($p<0.05$). Supercooling capacity is changing during the year and this is increasing when insect prepares for resting stage /Merivee, 1972/. Unseasonal cold in summer could cause a major mortality in freeze avoiding insects, because rapid cold hardening is insufficient time for such insects to clear their guts and undergo to the biochemical changes associated with the long-term acclimation for winter conditions (which is the process that may take days or weeks). By contrast, a species that exhibits some freeze-tolerance throughout the year could survive an unexpected freezing even without seasonal cold hardening, and with food in the gut, especially if it was of a relatively short duration and temperatures did not cross lethal thresholds /Sinclair et al., 2003/.

Conclusions

C. granulatus belongs to the cold-hardy freezing-tolerant insect group, but its supercooling capacity is weak. Overwintering could be harmful in cold winters without snow cover. *C. granulatus* can likely survive unseasonal cold.

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