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ACCUMULATION OF HIGH CONCENTRATIONS OF myo-INOSITOL IN THE OVERWINTERING LADYBIRD BEETLE *Ceratomegilla undecimnotata*.

Vladimír Košťál[1*], Oldřich Nedvěd[1,2] & Petr Šimek[1]

[1] Institute of Entomology, Academy of Sciences of the Czech Republic,
[2] Faculty of Biological Sciences, University of South Bohemia,
Branišovská 31, 370 05 České Budějovice, Czech Republic

ABSTRACT

The GC-MS analysis of the whole body samples of the overwintering coccinellid beetles *Ceratomegilla undecimnotata* revealed the presence of four compounds with potential cryoprotective role. There was no difference in the pattern of cryoprotectants between the sexes. The contents of glycerol, glucose and trehalose were relatively low and stable throughout the dormant state. The content of myo-inositol was low (2.5 μg/mg wet weight) in October, increased three-fold in November, peaked in February (11 μg/mg) and dropped again in spring.

Key words: *Ceratomegilla undecimnotata*, Coccinellidae, overwintering, supercooling, myo-inositol.

INTRODUCTION

Adult ladybird beetles *Ceratomegilla (*syn. *Semiadalia) undecimnotata* (Schneider, 1972) (Coleoptera: Coccinellidae) overwinter in large aggregations at the base of shrubs and in crevices in rocks on hills, usually above snow cover (Hodek, 1960), and are exposed to drastic fluctuations of air temperature and relative humidity during winter. Cold hardiness, in terms of supercooling capacity and survival at subzero temperatures, was studied in this species by Nedvěd (1993; 1994). Adult beetles did not tolerate spontaneous freezing of their body fluids even when the cooling/warming rates were relatively slow (10K/h) (Nedvěd, 1994). The supercooling point (SCP) was lower in diapausing beetles in late summer than in active beetles, decreased slowly to -19°C until mid-winter and remained low until spring. Good correlation has been found between the SCP value and the survival of 24 hrs exposure to subzero temperatures in this ladybird (Nedvěd, 1993; Nedvěd *et al.*, 1995). Water content remains constant, glycogen and lipid reserves are utilized during overwintering of *C. undecimnotata* (Hodek & Čerkasov, 1963).

Many overwintering insects accumulate low molecular weight sugars and/or sugar alcohols (Storey & Storey, 1991). These compounds protect both freeze-tolerant and freeze-avoiding organisms during freezing/thawing or prolonged exposures to low non-freezing temperatures, respectively (Zachariassen, 1985; Carpenter & Crowe, 1988). Harper & Lilly (1982) found that overwintering coccinellid *Hippodamia quinquesignata* accumulate only very low amounts of glycerol and traces of sorbitol. Parry (1986) found

no polyols in haemolymph of the overwintering ladybird *Aphidecta obliterata*. Bennet & Lee (1989) failed to detect any of the commonly reported cryoprotectants in concentrations greater than 1mM in the overwintering *Hippodamia convergens* and they concluded that it appears that coccinellids do not accumulate high concentrations of the commonly reported cryoprotective polyols and sugars and that additional studies are needed to determine what role, if any, low molecular weight polyhydric alcohols and sugars play in the cold hardiness of coccinellids. Hoshikawa (1981), however, reported the accumulation of inositol ranging between 1.5% and 1.9% of body weight in four species of overwintering coccinelids.

In this paper we recorded dynamics of low molecular weight cryoprotectants during overwintering of a ladybird *Ceratomegilla undecimnotata*. Two main aims of the study were: (1) to find potential correlations between accumulation of cryoprotectants, supercooling capacity and overwintering in this insect; and (2) to bring an additional information on the role of cryoprotectant accumulation in coccinellids.

MATERIALS AND METHODS

Overwintering beetles were collected from large aggregations on the slopes of volcanic Louny Hills in northern Bohemia. The samples were taken on three dates (3 September, 4 January, 5 May) during the winter 1993/94 and were moved to České Budějovice to an outdoor cage where the normal overwintering conditions were simulated. The ladybirds were kept in plastic boxes with moist sand and a vial with water. The pieces of corrugated cardboard were provided for sheltering. The temperature data presented in this study (Fig. 1) come from a meteorological station in České Budějovice. In order to verify the data, one additional sample was taken from the natural hibernacula at the turn of February and March 1996 and the contents of cryoprotectants were measured.

The subsamples of 25 - 30 overwintering beetles were taken (either directly from their natural overwintering sites or from an outdoor cage) monthly throughout the winter, were sexed, weighed and the determinations of the temperature of crystallization of body fluids (supercooling point, SCP) and of the contents of low molecular weight sugars and alcohols (cryoprotectants) were performed.

The SCP was measured by the method of Nedvěd *et al.* (1995) cooling the sample at the rate of 10K/h. Low molecular weight cryoprotectants were determined as their *o*-methyloxime trimethylsilyl derivatives and the identity of individual components was established against authentic standards by capillary gas chromatography coupled with mass spectrometry. The methods for preparation and processing of the samples were the same as described earlier (Košťál & Šimek, 1995; 1996).

RESULTS

Supercooling capacity

The SCPs of the whole body did not differ between the sexes ($P > 0.05$, Mann-Whitney U test) and thus the data were pooled for the presentation in Fig. 2. The mean SCP was intermediate (ca -14°C) in the autumn samples. Individual variability was, however, very high and some beetles showed low SCP (ca -20°C) already in October. The mean SCP dropped in early winter and remained low (ca -19°C) until the end of March. In May, when the overwintered beetles left their hibernacula, the SCP was high (ca -6°C), similar to the value known for active beetles (Nedvěd, 1993).

Cryoprotectants

The GC-MS analysis revealed the presence of four compounds with potential cryoprotectant role in the whole body samples of overwintering ladybirds. There was no clear difference in the pattern of cryoprotectants between the sexes. The contents of

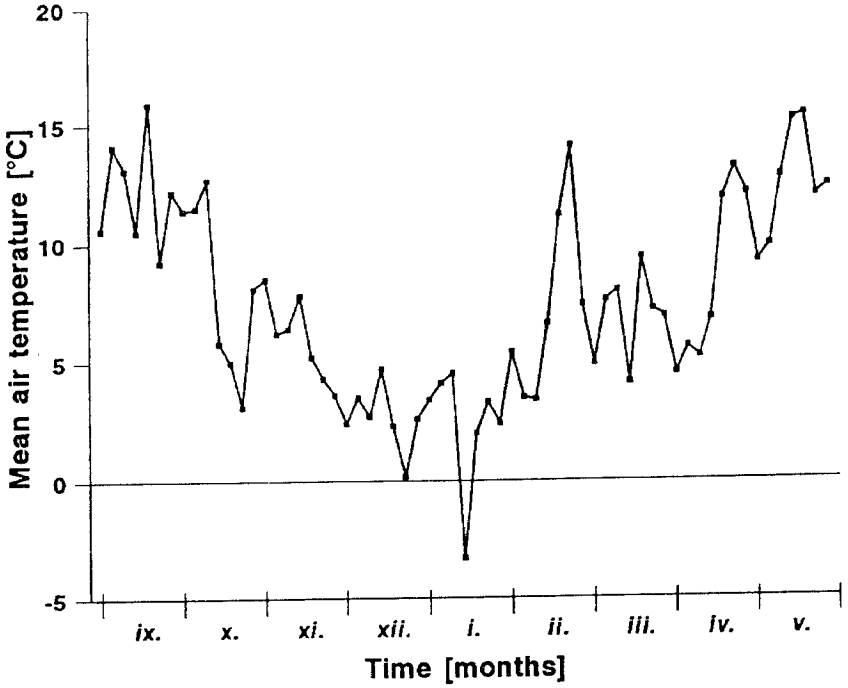

Fig. 1. Changes in the air temperature from September 1993 to May 1994. Each point represents mean air temperature in a pentad measured at the meteorological station in České Budějovice.

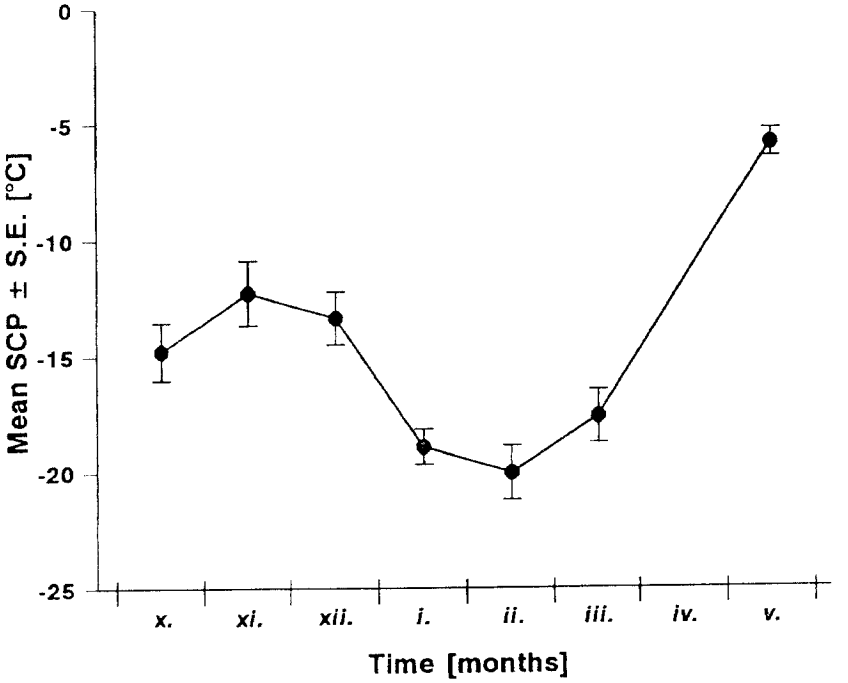

Fig. 2. Changes in the whole body supercooling point of the overwintering *Ceratomegilla undecimnotata* during the winter 1993/1994. Each point represents the mean of 8 males and 8 females.

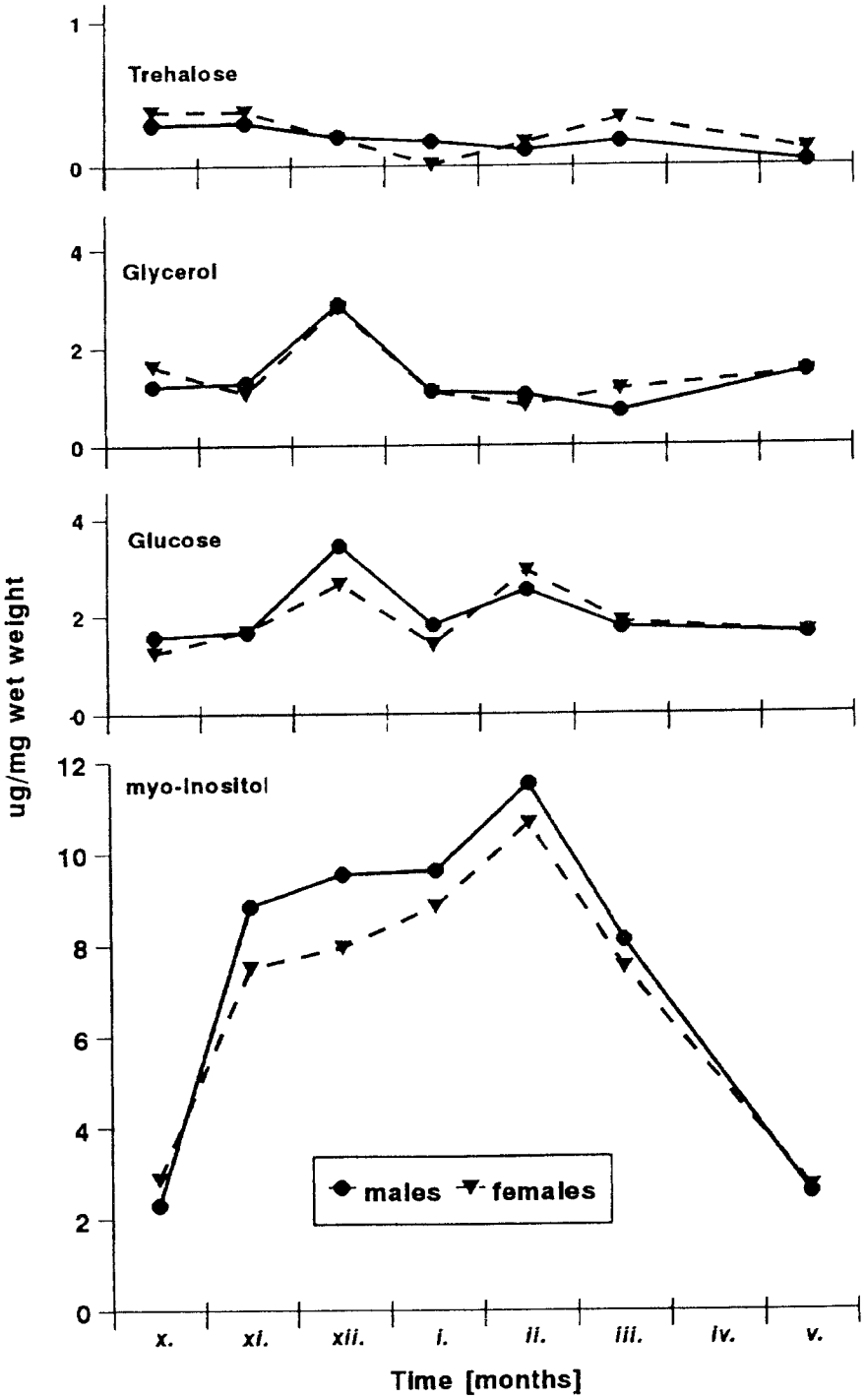

Fig. 3. Changes in the contents of cryoprotectants of the overwintering *Ceratomegilla undecimnotata* during the winter 1993/1994. Each point represents the value determined in a combined sample of 5 males or 5 females, respectively.

glucose, glycerol and trehalose were relatively low and stable throughout the winter 1993/1994 (Fig. 3). The content of myo-inositol was low (ca 2.5 μg/mg wet weight) in October, increased three-fold in November, peaked in February (ca 11 μg/mg) and dropped again in spring (Fig. 3). Analysis of one additional sample (3 males and 3 females) taken in 1996 confirmed the results obtained two years earlier. In this sample we found in females: 12.9 ± 4.8 (mean ± S.D.) μg/mg w.w. of myo-inositol; 3.4 ± 1.3 glucose; 0.4 ± 0.2 glycerol; 0.1 ± 0.1 trehalose; and in males: 16.9 ± 5.6 myo-inositol; 4.4 ± 2.5 glucose; 0.7 ± 0.3 glycerol; 0.5 ± 0.2 trehalose; 23.1 μg/mg was the highest content of myo-inositol recorded in one individual (male) ladybird.

DISCUSSION

In this paper we report accumulation of relatively high concentrations of myo-inositol during overwintering of a ladybird *Ceratomegilla undecimnotata*.

The sum concentrations of all cryoprotectants found in the ladybirds were too low, however, to protect the hibernating organisms through depression of melting point and supercooling point by colligative action (Zachariassen, 1985). This was also documented by a lack of synchrony between the decrease of the SCP (during January; Fig. 2) and the increase of myo-inositol content (already during November; Fig. 3). Moderate stabilizing effect of inositol on protein structure during freeze-thawing has been shown (Carpenter & Crowe, 1988). The ladybird *C. undecimnotata* overwinters, however, in a supercooled state and freezing is lethal for hibernating adults (Nedvěd, 1993; 1994). Whether inositol conveys any protective role to a supercooled ladybird remains to be elucidated.

Hoshikawa (1981) found the accumulation of inositol (without specification of its isomerism) to be a common phenomenon in four overwintering coccinellids and one chrysomelid beetle. In his study inositol was lost approximately when the beetles ceased hibernation and became active in the spring which was in a good accordance with our results (Fig. 3).

Accumulation of myo-inositol, in maximum concentrations ranging between 7 - 15 μg/mg w.w., either during overwintering or as a response to cold acclimation, has been documented for a few other arthropods including two sub-antarctic carabid beetles (Block & Sømme, 1983), three alpine oribatid mites and one collembolan (Schenker, 1983), one alpine carabid beetle (Bakken, 1985), and the house spider (Tanaka, 1995). In the above mentioned studies, myo-inositol always dominated and was usually accompanied by a mixture of various other sugars and polyols in much lower concentrations. The group of animals which accumulate preferentially myo-inositol during winter diapause or in response to cold acclimation is extremely heterogenous including species from relatively remote arthropod taxons and species inhabiting various climatic zones (Antarctic, alpine, temperate). The common feature for all these species, winter diapause, is typical for many other arthropods which accumulate high amounts of different cryoprotectants such as: glycerol (see Sømme, 1982; Lee, 1991 for references); trehalose (Hayakawa & Chino, 1982; Košťál & Šimek, 1995); sorbitol (Pullin *et al.*, 1991); ribitol (Hamilton *et al.*, 1985); ethylene glycol (Gehrken, 1985). Accumulation of glycerol during diapause was documented also in a tropical beetle *Stenotarsus rotundus* which never experience temperatures below zero in nature (Pullin & Wolda, 1993). Thus it seems that the accumulation of high concentrations of (so called) cryoprotectants is closely related to diapause but may be achieved also by cold acclimation. Both diapause and exposure to cold are connected with metabolic suppression which may be the actual cause for polyol and sugar accumulation (Pullin *et al.*, 1991).

The role and/or explanation for the presence of high concentrations of myo-inositol in an overwintering organism remain still unknown and requires further clarification.

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