

**Comparison of cold hardiness in two ladybird beetles (Coleoptera: Coccinellidae)
with contrasting hibernation behaviour**

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Abstract. The cold hardiness of two ladybird species from the Czech Republic, *Coccinella septempunctata* and *Semiadalia undecimnotata*, was measured in terms of their supercooling point (SCP) and survival at subzero temperatures. The SCP was lower in diapausing beetles in late summer than in active beetles, and the SCP of diapausing beetles decreased slowly until mid-winter. The SCP of *S. undecimnotata*, which overwinters exposed to air, was lower (to -19°C), that of *C. septempunctata*, which overwinters at ground level insulated in plant material, was higher (to -15°C). The SCP of *C. septempunctata*, exposed to extreme fluctuations of temperature in an outdoor insectary, decreased to -21°C . After the termination of diapause, the SCP of *C. septempunctata* from natural hibernacula increased in the later part of the cold period. In contrast, the SCP of *S. undecimnotata* and *C. septempunctata* in an outdoor insectary remained low until spring. The cost of being more cold-hardy is possibly compensated for by a low mortality from fungal disease.

INTRODUCTION

Insects survive winter frosts because they can tolerate freezing or avoid freezing by supercooling (e.g. Bale, 1989). Although there are doubts about the importance of supercooling for survival (e.g. Bennett & Lee, 1989), the supercooling point (SCP) is considered to be a good indicator of cold-hardiness in many insect taxa (Bale, 1989).

Different microhabitats are used for hibernation by Coccinellidae (Hodek, 1960; 1973). Lee (1980) compared the adaptations to hibernation conditions in two Nearctic ladybirds: *Hippodamia convergens*, which overwinters in large aggregations in the mountains, and *Coleomegilla maculata*, which overwinters in litter beneath snow. The SCP of *H. convergens* remained low for several days or even weeks after transfer to 20°C , while in *C. maculata* the SCP rose within a few days of the transfer.

Similarly, European ladybirds are known to use different sites for hibernation, either insulated or exposed. A much higher resistance to low temperature as well as to low moisture was found in *Adalia bipunctata*, which hibernates in bark crevices, than in *Coccinella septempunctata*, *C. quinquepunctata* and *Exochomus quadripustulatus*, which hibernate in litter (Novák & Grenarová, 1967). This study concentrated on monitoring the supercooling ability over the whole non-reproductive period with the aim of determining whether the SCP is related to overwintering behaviour.

MATERIAL AND METHODS

CHARACTERISTICS OF THE SPECIES. For monitoring the changes in cold-hardiness during the non-reproductive period, two species of ladybirds available in large numbers from hibernation quarters were used. *Coccinella septempunctata* L. overwinters in several types of hibernacula, always insulated – “subnival” in the sense of Lee (1980): in grass tussocks and under dead leaves (Hodek, 1960; Honěk, 1989).

Hippodamia (Semiadalia) undecimnotata (Schneid.) overwinters in large aggregations at the base of shrubs and in crevices in rocks on hills, usually above snow cover (Hodek, 1960).

CHARACTERISTICS OF THE HIBERNATION SITES. The ladybirds were sampled from hibernation sites at four localities:

Locality A. A forest edge near the village Kamenný Újezd, southern Bohemia. *C. septempunctata* hibernated at the foot of a small hill, at 400 m a.s.l., with S and SW facing slope. Samples from this locality were measured over the whole dormancy period (i.e. September 1990 to May 1991), as beetles could be collected even in winter if the snow cover was not continuous. The SCP was measured within a few days of collecting the adults.

Locality B. Large aggregations of dormant *Semiadalia undecimnotata* and *C. septempunctata* can be found on the hill Raná (457 m a.s.l.) in the volcanic Louny hills, northern Bohemia (Hodek, 1960). *S. undecimnotata* overwinters at the base of shrubs and in rock crevices on the SW slope. Large samples of this species were collected on October 16, 1990 and October 13, 1992. These beetles were kept in an outdoor insectary and in 1992 also in a hole filled with leaf litter, and subsamples were used for measurements.

Locality C. *C. septempunctata* overwinters in leaf litter in a large clearing on the mild northern slope of a hill near the town Mladá Boleslav, central Bohemia, 300 m a.s.l. The northern facing slope is an unusual site for ladybirds to migrate to (Honěk, 1989). Beetles were collected from this site on November 23, 1990 and placed in an outdoor insectary.

Locality D. A large sample of *C. septempunctata* was collected on September 3, 1992 from grass tussocks growing among limestone rocks on W slope of the hill Děvín in Pavlov hills, southern Moravia, and placed in an outdoor insectary and the hole described below.

CONDITIONS OF STORAGE. Samples from the field were placed in an outdoor insectary to simulate the extreme conditions *S. undecimnotata* is exposed to in its hibernacula. The ladybirds were kept in plastic boxes with either moist sand and a vial with water or with dry sand. Another sample was placed in a hole burrowed in the ground, partially filled with leaf litter to simulate the *C. septempunctata*-type insulated hibernacula. Only the storage on moist sand was used.

Actively reproducing specimens were obtained from a culture kept at 20°C, 18L:6D, and fed with *Acyrtosiphon pisum*, and from the field in early summer.

The temperature data used in this paper were obtained from a meteorological station in České Budějovice where the mean temperature is 8.3°C (season 1990–91), and from a thermohygrograph in the outdoor insectary and the hole (season 1992–3).

SUPERCOOLING POINT AND SURVIVAL. The SCP was measured using a copper-constantan thermocouple kept in tight contact with the insect body as described by Brunnhofer et al. (1991), connected to a recorder. A cooling rate of 1–3°C/min was manually controlled. Handling the ladybirds evoked reflex bleeding, which might cause inoculative freezing at a higher temperature. Before placing a ladybird in a syringe, the beetle was provoked to bleed and the droplets of haemolymph removed.

SCP of ladybirds was measured at more or less regular intervals from late summer, when migration to the hibernation sites began, to spring, when overwintered ladybirds dispersed. All SCP values are means of measurements on 6 to 54 individuals, most often 18.

Survival after exposure to subzero temperatures was measured in two ways (Nedvěd & Hodková, in prep.): 1) by exposure of the ladybirds for 24 h to series of low temperatures and counting the number alive after another 24 h; 2) by cooling a sample of ladybirds until half of them froze, and then immediately warming them up and counting mortality 24 h later. Measurements of survival were made over the periods September to October, December to January and March to April in both species.

RESULTS

Importance of supercooling for survival

The temperature at which 50% of the dormant ladybirds survived was always equal to or lower than the median SCP. The supercooling ability of reproducing beetles offers them some protection from freezing. Post-dormant individuals, transferred in January to 21°C and 18L:6D and fed with *Acyrtosiphon pisum*, increased its SCP within three weeks

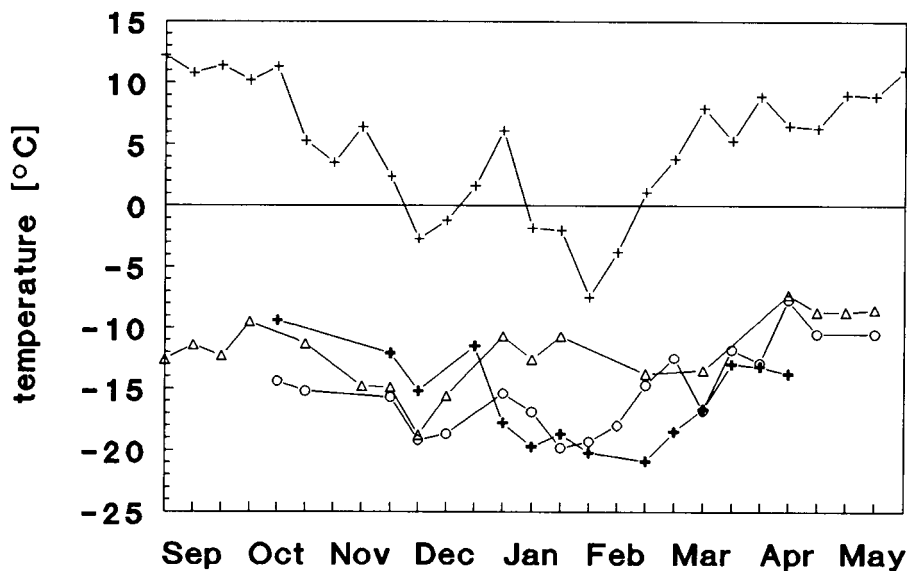


Fig. 1. Changes in the supercooling point and ambient temperature from September 1990 to May 1991. Light crosses – mean ambient temperature, triangles – *Coccinella septempunctata* from natural hibernacula, open circles – *Semiadalia undecimnotata* from the insectary, heavy crosses – *Coccinella septempunctata* from the insectary.

(from -11°C to -7.5°C in *C. septempunctata* and from -15°C to -9.5°C in *S. undecimnotata*). The temperature at which 50% survived increased similarly. The warm-acclimated beetles survived three days at -5°C , and laid viable eggs within one week; they exceptionally survived -10°C .

Changes in the SCP during the non-reproductive period

The SCP of *C. septempunctata* from natural hibernacula and from insulated conditions (hole) decreased from autumn to early winter, but not too much (Figs 1 and 2). In late winter, after diapause termination, the SCP even increased although hard frosts occurred above the snow layer in February. In March or April, when ladybirds left the hibernacula, the SCP increased to the value observed in active beetles.

The seasonal pattern of changes in SCPs differed in *C. septempunctata* kept in the outdoor insectary (Figs 1 and 2). They were similar to those in the natural hibernation sites only to early winter. In mid-winter, however, in spite of periods of decreases and increases in temperature, the SCP of the insectary beetles decreased substantially. The SCP increased during the warm spells in March but in April the mean SCP was still much lower than in active ladybirds.

The SCP of *S. undecimnotata* placed in the insectary in October was much lower than that of active individuals (-15°C vs. -7°C), decreased to mid-winter, and remained low for a long time even in ladybirds that had emerged from dormancy in spring (Figs 1 and 3). The changes in SCP are roughly correlated with the fluctuations in air temperature over this period.

The SCP of *S. undecimnotata* from insulated storage remained relatively high for a long time and then dropped in early winter and remained low until March, as in the insectary beetles.

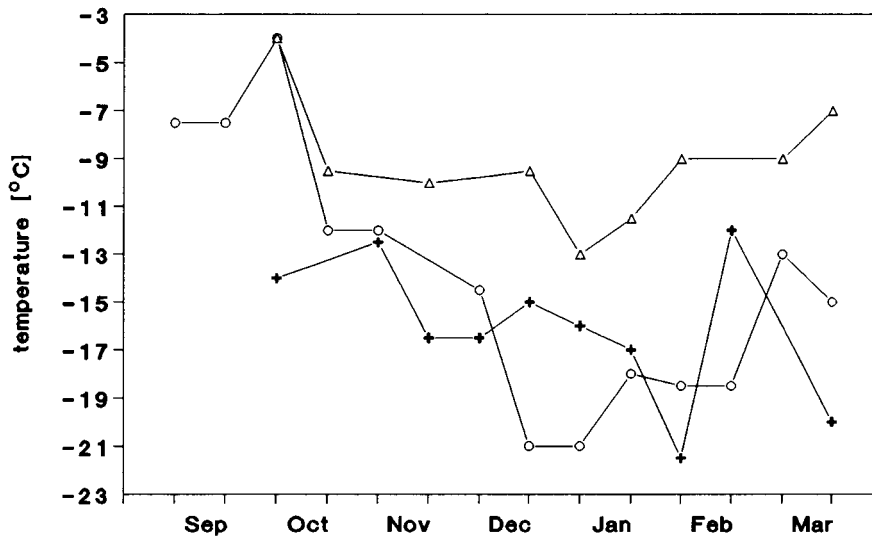


Fig. 2. Changes in the supercooling point from September 1992 to March 1993 of *Coccinella septempunctata*. Triangles – samples from insulated hibernacula, circles – outdoor insectary, moist conditions, crosses – outdoor insectary, dry conditions.

Hibernation ethology

Adults of *C. septempunctata* arrived at dormancy sites from July to September and formed small aggregations on plants together with other ladybird species (*C. quinquepunctata*, *Adonia variegata* et al.). They moved from plants to the insulated hibernacula in moist decaying plant material gradually over the period August to December (!), and in the

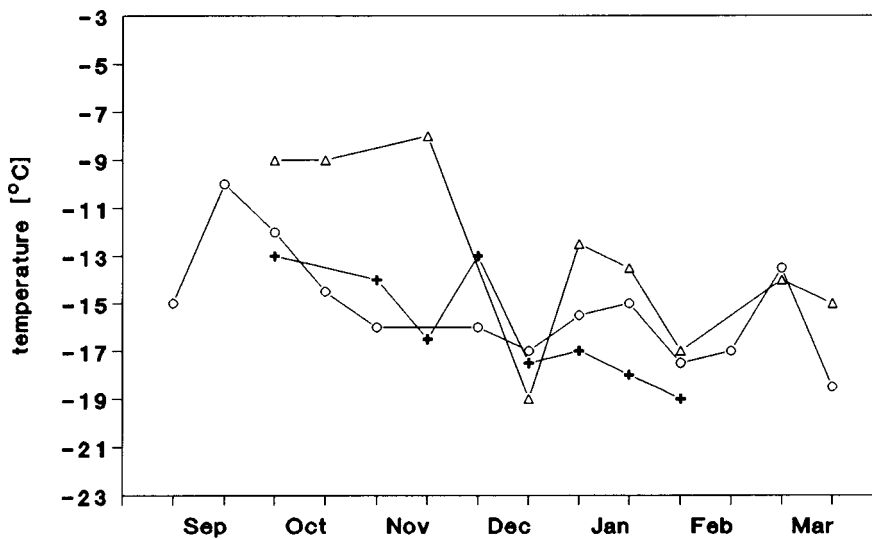


Fig. 3. Changes in the supercooling point from September 1992 to March 1993 of *Semiadalia undecimnotata*. Triangles – samples from insulated hibernacula, circles – outdoor insectary, moist conditions, crosses – outdoor insectary, dry conditions.

warmer periods of late winter, even as early as late January (!), they left the insulated hibernacula and basked in the sun. They dispersed from the dormancy site from March to April.

S. undecimnotata arrived at its dormancy sites in July and August and soon formed large aggregations at the base of shrubs and in rock crevices. They left their hibernacula in April and dispersed.

Mortality of the beetles kept in the insectary was about 20% in *C. septempunctata*, and 5–10% in *S. undecimnotata* through March, similar to that observed in the field. However, the ladybirds in the insulated hole experienced a high mortality: almost 100% in the former species, and 50% in the latter.

DISCUSSION

The two ladybird species differed in their SCP values and patterns of seasonal changes in supercooling when hibernated in their natural conditions.

Importance of supercooling for survival

In some species, there is a weak or no correlation between the SCP and the lower lethal temperature (Bennett & Lee, 1989). In contrast, we have found a good correlation between the survival at subzero temperatures and the SCPs in the two ladybird species.

The pattern in changes in the SCP of *S. undecimnotata*, during and after diapause, is similar to those shown by *Hippodamia convergens* (Lee, 1980). Postdiapause *H. convergens*, however, lose the ability to survive subzero temperatures after warm-acclimation (Bennett & Lee, 1989). *S. undecimnotata* retained this ability and survived subzero temperatures slightly above its SCP. This probably enables this species, which usually hibernates in rock crevices, to survive late frosts in spring. *S. undecimnotata* leaves its dormancy sites much later than other ladybirds; part of the population often remains there until mid-May (Hodek, 1960).

Relation of cold hardiness to the type of hibernacula

The often recorded general ecological plasticity of *C. septempunctata* (Hodek, 1973) was corroborated by the ability to modify the pattern of SCP changes when exposed to ambient winter temperature. The conditions in the insectary are usual for *S. undecimnotata*, but not for *C. septempunctata* when insulated in their typical hibernacula. Exposure to very low temperatures (or its great amplitudes) resulted in a greater decrease in the SCP, than observed in *S. undecimnotata*. However, the opposite was not observed as the SCP in *S. undecimnotata* decreased even when it hibernated insulated in the hole alongside with *C. septempunctata*.

The values and pattern of change in SCP seemed to be similar in dry and moist insectary conditions (Figs 2 and 3). Thus we assume that the difference in SCP between the two types of storage was simply due to the different ambient temperatures.

The supercooling ability of *S. undecimnotata* in winter was similar (-19°C) to that of *H. convergens* and *C. maculata* (Lee, 1980). As in *H. convergens*, the SCP in *S. undecimnotata* remained low for a long time after exposure to high temperature. In contrast, the SCP of *C. septempunctata* was higher and its supercooling ability did not decrease so rapidly as that of *C. maculata*, although *C. septempunctata* uses similar microhabitats for overwintering to the Nearctic species.

The two pairs of species differ in their sensitivity to low humidity. *S. undecimnotata*, which is exposed to the air and ambient humidity when overwintering, and *H. convergens*

are better able to tolerate low ambient humidity at both high and low temperatures. *C. septempunctata* and *C. maculata*, overwintering in grass tussocks, litter and soil, are sensitive to low humidity. This is supported by the observation of Novák & Grenarová (1967) that *C. septempunctata* and *E. quadripustulatus* survive well only when insulated by snow from the ambient air temperature and humidity. *S. undecimnotata* overwinters in non-insulated sites and has to be able to tolerate low humidity and very cold conditions but has the advantage of a low infection by fungus (mycosis). If after migrating to the dormancy sites *S. undecimnotata* fails to find an optimal site in a rock crevice and has to cluster at the base of a shrub or in a similar moist microhabitat, mortality from mycosis is very high (Iperti, 1966). *C. septempunctata* probably decreases the risk of mycosis, which may occur frequently in wet decaying plant material, by entering hibernacula late in autumn and leaving them early in spring, and by overwintering in small aggregations.

Beetles from different localities and different years did not differ in their SCP pattern or in their general overwintering behaviour.

The SCP was lower in ladybirds collected in early autumn, before arriving of frosts, than in active beetles. Similarly to *Pyrrhocoris apterus* (Hodková & Hodek, 1993), cold hardiness in Coccinellidae appears to be part of their "diapause syndrome" (De Wilde, 1970). Supercooling ability can, however, be increased by decrease in ambient temperature. As the diapause syndrome "survives" diapause, supercooling is retained even after the termination of diapause.

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