

## Overwintering of *Coccinella septempunctata* (Coleoptera: Coccinellidae) at different altitudes in the Karkonosze Mts, SW Poland

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**Abstract.** The body length variation, sex ratio, ovarian development and natural enemies (parasitoids and entomopathogenic fungi) of *Coccinella septempunctata* were studied during two dormancy seasons in three hibernation sites in the Karkonosze mountains: the top of Mt. Śnieżka (1,600 m a.s.l.), the top of Mt. Szrenica (1,360 m a.s.l.) and Karpacz, the village at the foot of Mt. Śnieżka (800 m a.s.l.). The proportion of females and mean body length increased with the altitude of the hibernaculum. Post-diapause maturation of ovaries occurred earlier in spring in females from Karpacz than from the mountain tops. The rate of parasitization of *C. septempunctata* by its most common parasitoid, the braconid *Dinocampus coccinellae*, in both seasons exceeded 70% at Karpacz and was 14–28% in the mountain top hibernacula. In contrast, the incidence of fungal infection (mainly by *Paecilomyces farinosus* and *Beauveria bassiana*) was higher in beetles overwintering on the two mountain tops.

### INTRODUCTION

The seven spot ladybird, *Coccinella septempunctata* L., spends the winter dormancy period in a variety of habitats. Beetles can hibernate near their breeding sites: at forest edges, clearings, wind breaks, near isolated shrubs or can migrate to the hibernacula situated on the summits of prominent hills or mountains (Honěk, 1989; Hodek et al., 1993; Hodek & Honěk, 1996). Migrants and individuals remaining for winter in the proximity of breeding sites differ in some biological and ecological characters such as the intensity of diapause, duration of the dormancy period, female/male ratio, fat reserves or mean body size (Hodek et al., 1977; Honěk, 1989; Zhou et al., 1995). While relatively large, fatty and predominantly female individuals arrive at the prominent dormancy sites during August to hide among the stones or in grass tussocks, the smaller ones, staying at lower situations, can be found on plants even in October (Honěk, 1989). Resumption of mobility after dormancy is dependent on ambient temperature (Hodek, 1960; Hodek & Honěk, 1996), and is thus related to the position of the dormancy site; individuals hibernating under severe conditions of mountain peaks disperse later.

Many assumptions concerning the adaptive significance of coccinellid migration to prominent landscape features have been proposed (see Hodek, 1960 and Hodek & Honěk, 1996, pp. 266–267). For example, Semyanov (1965) suspected that the tendency to hibernate in elevated places may enable the coccinellids to escape from spring flooding, whereas Honěk (1989) emphasized the importance of the selection of habitats with appropriate temperature/humidity relationship and sufficient aeration. The latter author argued that since many individuals entering hibernacula are infected by pathogenic microorganisms, overwintering under conditions favourable for

the spreading of diseases may be disastrous for coccinellids.

The main purpose of this study was to test the hypothesis that overwintering at high elevations can reduce *C. septempunctata* mortality caused by natural enemies (parasitoids and entomopathogenic fungi). Thus, the parasitization rates of the beetles spending the dormancy period in hibernacula at different elevations and the presence of entomopathogenic Hyphomycetes (Fungi imperfecti) in dead beetles collected from those hibernacula were determined. Furthermore, post-hibernation mortality accompanied by fungal infection or parasitoid emergence was assessed experimentally. To check previously reported relations between ecological and physiological parameters of overwintering coccinellids and the position of hibernation site, body size, sex ratios and the state of females' ovaria were also determined.

### MATERIAL AND METHODS

#### Characteristics of *C. septempunctata* overwintering at different elevations

Samples of *C. septempunctata* from two montane hibernation sites (the top of Mt. Śnieżka, 1,600 m a.s.l., 50°44'N, 15°45'E and the top of Mt. Szrenica, 1,360 m a.s.l., 50°47'N, 15°31'E) and from the site at the foot of Mt. Śnieżka (Karpacz village, about 800 m a.s.l., 50°46'N, 15°45'E) were taken in late summer/autumn and in spring (soon after the disappearance of snow cover from the mountain tops) within two dormancy seasons, 1991/92 and 1992/93. The length of each beetle was measured and its sex, parasitization by parasitoids and, in females, developmental stage of ovaries were determined by dissection. Parasitoids found in the beetles were identified at least to the family level and their developmental instars recorded. The maturation level of coccinellid ovaries was evaluated with the 5-degree scale, from 1 (ovarioles without oocytes) to 5 (chorionized eggs) (see Okuda & Hodek, 1989).

### Entomopathogenic fungi found on dead *C. septempunctata* in hibernacula

Dead *C. septempunctata* found at the hibernation sites were checked for infestation by entomopathogenic Hyphomycetes. The growth and sporulation of the fungi on infected cadavers were stimulated by placing them in humid chambers (Petri dishes with moist filter paper) at 25°C. Fungal pathogens were then identified either directly on insects covered by sporulating mycelium or in preparations made from purified cultures grown on solid artificial media (Sabouraud's glucose medium and water agar) (Stockdale, 1971).

### Experimental analysis of post-hibernation mortality

Mortality rates of *C. septempunctata*, the rates of parasitoid emergence from the beetles and the rates of infection by fungal pathogens after overwintering in two types of dormancy sites were assessed. On May 26–27, 1992, samples of 100 individuals from the top of Mt. Śnieżka and from its foot (Karpacz) were taken. The beetles were then kept outdoors in rearing containers with water and honey solution supplied. At 2- to 4-day intervals coccinellid mortality and parasitoid emergence were recorded and dead coccinellids removed from the containers. The rearings lasted until all beetles died. After dying insects were checked for infestation by entomopathogenic fungi according to the procedure described above.

## RESULTS

### Sex ratio, size and ovaries

A clear correlation between altitude of dormancy site and sex ratio, as well as the size of both female and male coccinellids was found, especially in autumnal samples (Table 1). The tendency of the mean body length to increase with altitude of hibernaculum site was statistically significant for the females collected in autumn 1991 (ANOVA,  $F_{2,281} = 7.99$ ,  $P < 0.001$ ), in autumn 1992 ( $F_{2,194} = 11.79$ ,  $P < 0.001$ ) and in spring 1993 ( $F_{2,177} = 11.20$ ,  $P < 0.001$ ). For males, the differences were less distinct and were significant only in autumn 1991 ( $F_{2,188} = 6.50$ ,  $P < 0.002$ ).

All females sampled in September 1991 and September 1992 from both montane sites had arrested ovarian development. However, maturation of ovaries was slightly advanced in a few females taken from Karpacz in Sep-

tember 1992. Surprisingly, one female collected from this locality in November 1991 possessed mature eggs. In spring samples, the great majority of females from Mt. Śnieżka and Mt. Szrenica had ovaria at stage 1, while oogenesis was already in progress in many females from Karpacz (Table 1).

### Parasitoids

The only parasitoid frequently found in dissected beetles was *Dinocampus coccinellae* Schrank (Hymenoptera: Braconidae). Nematodes (Mermithidae) were recorded in 3 beetles (2 in September 1991 and 1 in May 1992) from Karpacz and dipterans (Tachinidae), in 2 beetles (both in May 1992) from the same locality.

Although *D. coccinellae* is a solitary parasitoid, in many coccinellids more than one egg and/or 1st instar larva was recorded (in individual samples the superparasitism rate ranged between 22 and 64%). However, just one larva can survive to the 2nd instar. The rate of total parasitization of the samples, recorded as the proportion of beetles containing any dead or living *D. coccinellae* individual, was divided into 3 groups (Fig.1): (1) hosts with living eggs and/or 1st instar larvae, i.e. the stages in which the parasitoid can diapause, (2) hosts with living 2nd or 3rd, i.e. certainly not diapausing, larvae and (3) coccinellids containing only dead parasitoids at any instar. The third component comprised up to 21% of the total parasitized.

In autumn, the total rate of parasitization of *C. septempunctata* collected in Karpacz was about three times higher than that of those from montane hibernacula and in both years it exceeded 70%. In September 1992, the development of parasitoids was not arrested in 16% of parasitized coccinellids from Karpacz.

In the subsequent spring samples, the differences in the parasitization rate between mountain foot and mountain top sites were smaller. However, at the spring sampling dates, post-diapause larval development of *D. coccinellae* in Karpacz was considerably advanced and in many cases already completed.

TABLE 1. Sex ratio, mean body length and the state of ovarian development in *C. septempunctata* sampled from different hibernation sites.

Date of sampling	Dormancy site	No. females	No. males	Female/male ratio	Length (mm) (mean ± SD)*		No. females with ovaria at the stage:				
					Females	Males	1	2	3	4	5
Sept. 8–9 and	Mt. Śnieżka	96	27	3.56	7.30 ± 0.34 a	6.79 ± 0.30 a	96	–	–	–	–
Nov. 9, 1991**	Mt. Szrenica	49	28	1.75	7.15 ± 0.35 b	6.69 ± 0.28 a,b	49	–	–	–	–
May 26–27, 1992	Karpacz	139	136	1.02	7.11 ± 0.39 b	6.57 ± 0.32 b	138	–	–	–	1
	Mt. Śnieżka	64	31	2.06	7.18 ± 0.38 a	6.66 ± 0.28 a	60	4	–	–	–
Sept. 18–19, 1992	Karpacz	58	47	1.23	7.06 ± 0.35 a	6.61 ± 0.34 a	13	12	33	–	–
	Mt. Śnieżka	77	28	2.75	7.35 ± 0.34 a	6.78 ± 0.36 a	77	–	–	–	–
	Mt. Szrenica	74	33	2.24	7.31 ± 0.31 a	6.65 ± 0.35 a,b	74	–	–	–	–
May 18–20, 1993	Karpacz	46	52	0.88	7.05 ± 0.42 b	6.59 ± 0.40 b	43	3	–	–	–
	Mt. Śnieżka	73	31	2.35	7.42 ± 0.30 a	6.67 ± 0.39 a	72	1	–	–	–
	Mt. Szrenica	55	51	1.08	7.30 ± 0.32 b	6.72 ± 0.37 a	54	1	–	–	–
	Karpacz	52	47	1.11	7.13 ± 0.39 c	6.60 ± 0.34 a	37	12	3	–	–

\*Data marked by different letters within a given date of sampling/sex combination indicate a significant ( $P < 0.05$ ) difference in mean body length (LSD test).

\*\*Apart from the samples taken in September, an additional sample was taken in Karpacz in November 1991. Data of two samples from Karpacz, as they were not significantly different, are pooled.

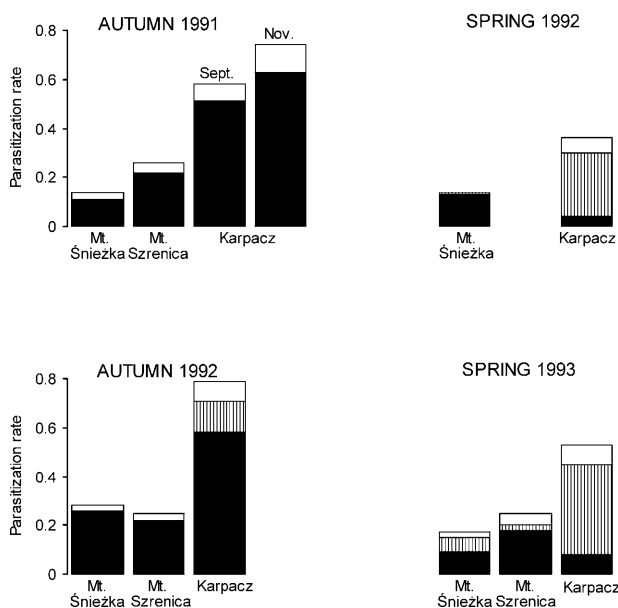


Fig. 1. Rates of parasitization by *Dinocampus coccinellae* of *Coccinella septempunctata* overwintering in different hibernation sites. Solid part of the bars – hosts containing living eggs and/or first instar larvae of *D. coccinellae*. Lined part of the bars – hosts containing living 2nd or 3rd instar larvae of *D. coccinellae*. Open part of the bars – hosts only containing dead *D. coccinellae* individuals in any instar.

### Entomopathogenic fungi on field collected cadavers

While plenty of dead coccinellids could be found at both montane hibernation sites, it was difficult to find any at Karpacz. Thus, the incidence of entomopathogenic Hyphomycetes in field collected coccinellid cadavers is only given for the samples from Mt. Śnieżka and Mt. Szrenica (Fig. 2). The fungal pathogens were identified as *Beauveria bassiana* (Balsamo) Vuillemin, *Paecilomyces farinosus* (Dicks) Brown & Smith, *Verticillium lecanii* (Zimmermann) Viégas and *Cephalosporium* sp. *V. lecanii* and *Cephalosporium* sp. were evaluated by laboratory tests as being very weak pathogens of *C. septempunctata*, in contrast to the very virulent *B. bassiana* and the intermediately virulent *P. farinosus* (Ceryngier & Hodek, 1996; E. Mierzejewska & P. Ceryngier, unpubl. data). Due to this, numbers of the first two taxa were pooled. The incidence of weak pathogens was higher and of the most virulent pathogen (*B. bassiana*) lower on the beetles collected from Mt. Śnieżka than from Mt. Szrenica. The total incidence of the two more virulent species (*B. bassiana* and *P. farinosus*) was 50% and 69% for Mt. Śnieżka and Mt. Szrenica, respectively. In both localities, mixed infections, i.e. the occurrence of more than one fungal entomopathogen on the same beetle, were common.

### Post-hibernation mortality

At the beginning of the rearings, the mortality rate of coccinellids from Karpacz was higher than that of coccinellids from Mt. Śnieżka, mainly due to the emergence of *D. coccinellae* larvae from parasitized individuals (Fig. 3A). Between late June and late July, the population from

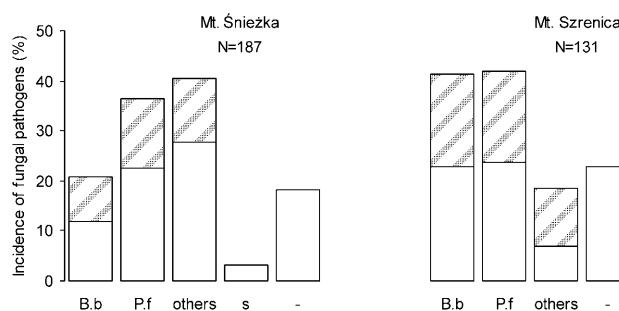


Fig. 2. Incidence of entomopathogenic Hyphomycetes on field collected *Coccinella septempunctata* cadavers. B.b – *Beauveria bassiana*; P.f – *Paecilomyces farinosus*; others – other entomopathogenic Hyphomycetes (incl. *Verticillium lecanii* and *Cephalosporium* spp.); s – sterile (not sporulating) mycelium; – = no entomopathogenic hyphomycete found; diagonally striped part of the bars – contribution of the fungus to mixed infections. Note that the sum of the values of individual bars exceeds 100% due to mixed infections

Mt. Śnieżka declined faster than the population from Karpacz. Only a few individuals from both localities survived till the beginning of August. All beetles of Karpacz origin soon died while those from Mt. Śnieżka persisted till mid-September.

Four percent of ladybirds from Mt. Śnieżka and 24% from Karpacz died as a result of the emergence of *D. coccinellae* (Fig. 3B). Apart from those beetles (not tested for the infestation by fungal pathogens), all remaining beetles were infected by entomopathogenic Hyphomycetes. However, the majority of the fungi found on ladybird corpses belonged to the group of weak *C. septempunctata* pathogens. Both of the more virulent pathogens, *B. bassiana* and *P. farinosus*, were about twice as frequent on the beetles from Mt. Śnieżka than from Karpacz. Additionally, mixed infections were more often detected on insects originating from Mt. Śnieżka (Fig. 3C).

## DISCUSSION

### Quality of low and high altitude hibernators

This study confirmed the earlier finding of Honěk (1989) that montane hibernacula of *C. septempunctata*, situated far from the breeding areas, are occupied by larger, predominantly female, individuals. Larger individuals are usually more vigorous and larger females more fecund than smaller ones (Rhamhalinghan, 1985; Honěk, 1993). Thus, ladybirds overwintering on the mountain tops appear to constitute the superior part of the population to those spending dormancy near to the breeding sites. According to Honěk (1989), the latter are often unable to perform long-distance migration due to their small size and lower metabolic reserves.

### Impact of entomopathogenic fungi on low and high altitude hibernators

Iperti (1966) found that in a coccinellid, *Ceratomegilla undecimnotata*, the incidence of mycosis caused by *Beauveria bassiana* was much lower when the beetles overwintered in more elevated hibernacula. In contrast,

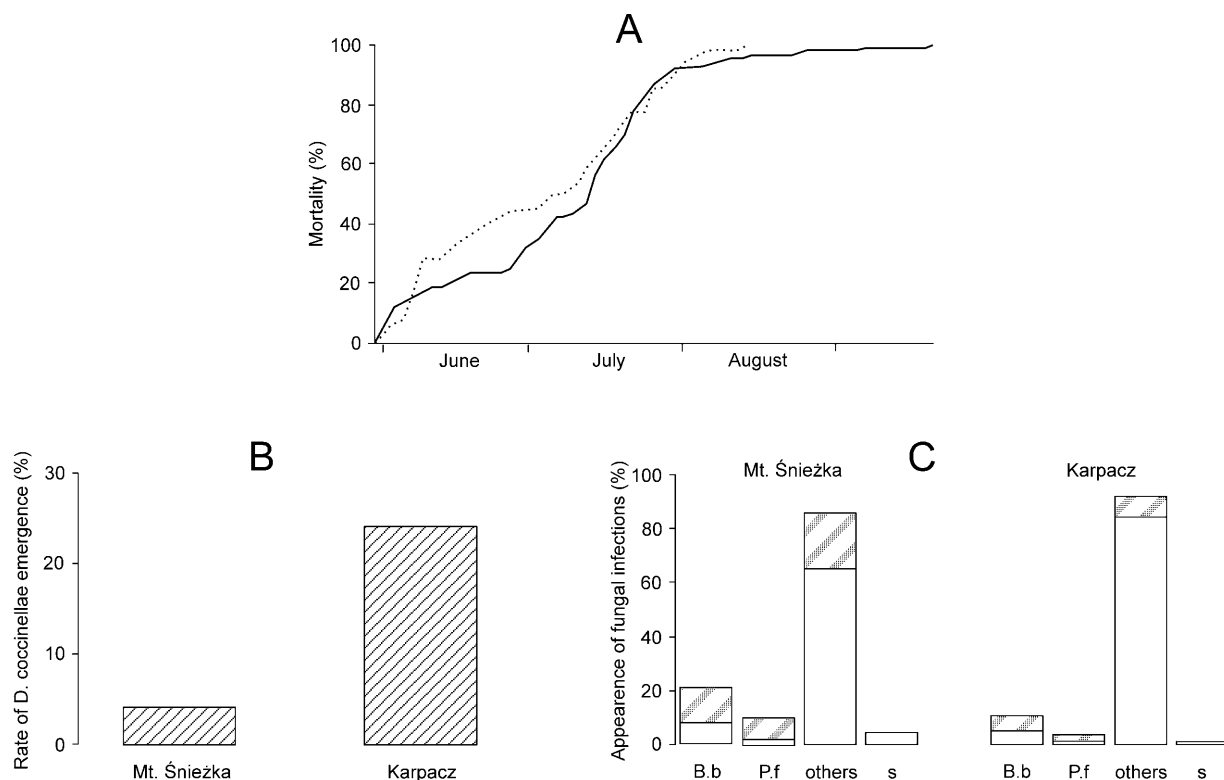


Fig. 3. Mortality and possible mortality factors of *Coccinella septempunctata* in post-dormancy rearings. A – mortality of *C. septempunctata* sampled on the top of Mt. Śnieżka (solid line) and in Karpacz (dashed line); B – rates of emergence of *Dinocampus coccinellae* from *C. septempunctata*; C – appearance of fungal infections on dead beetles (symbols as in Fig. 2).

our study suggests that *C. septempunctata* beetles overwintering on the mountain tops suffer heavy mortality from the mycoses. The high number of dead adults found at both montane hibernacula and the very frequent occurrence of fungal entomopathogens on those cadavers (often more than one entomopathogen on the same cadaver) indicate that the mountain tops were areas of extensive epizootics.

Overwintering coccinellids infected by highly virulent pathogens like *B. bassiana* usually do not survive the hibernation period. Mortality caused by *B. bassiana* is especially high in the late phase of dormancy, in early spring (Lipa et al., 1975; Mills, 1981). It is, thus, not surprising that the individuals which died in post-hibernation rearings were mostly infected by relatively weak pathogens (*V. lecanii* and *Cephalosporium* sp.). Infection by more virulent species, *B. bassiana* and *P. farinosus*, although rarely detected in the post-hibernation period, was more frequent in coccinellids from the montane hibernaculum than from that situated at the foot of the mountain.

#### Parasitization rates of low and high altitude hibernators: possible source of differences

About three times lower rate of parasitization of *C. septempunctata* by *D. coccinellae* in the montane hibernacula than in Karpacz may suggest that the adaptive significance of overwintering at higher elevations is the reduced risk of attack by this parasitoid. However, the effect of elevation on the parasitization rate is presumably

indirect. The observed differences may be derived from the different physiological and behavioural states of montane and low altitude ladybirds. Certainly, among beetles aggregating in late summer and autumn in both types of hibernacula some are parasitized by *D. coccinellae*. The parasitoids may either enter diapause (typically as the 1st instar larvae, rarely as eggs) together with their hosts or may promptly develop to the adult stage in hosts which are still not dormant (Hodek, 1973; Ceryngier & Hodek, 1996). The “autumnal” *D. coccinellae* females, emerging in the sites of high ladybird densities, might be expected to easily find and parasitize many hosts. However, the effectiveness of foraging *D. coccinellae* in such aggregations depends on the availability of hosts which are still mobile. Dormant ladybirds, hidden in the hibernation shelters, are inaccessible for *D. coccinellae* (Balduf, 1926; Walker, 1961; Richerson & DeLoach, 1972). As *C. septempunctata* entering montane hibernacula are characterized by deep diapause and early onset of dormancy, (1) parasitoid development in these beetles is arrested and (2) the availability of hosts for the foraging parasitoid adults is limited. In contrast, *C. septempunctata* individuals which do not perform long-distance migrations remain active much longer. This leads to the development of *D. coccinellae* within autumnal aggregations of those beetles (in some *C. septempunctata* from Karpacz *D. coccinellae* development was still not arrested in September) and, subsequently, to the efficient foraging of a new generation of *D. coccinellae* females (the parasitization rate of coccinellids in Karpacz significantly increased between

September and November, 1991) (Fig. 1). Thus, the principal behaviour of *C. septempunctata* which may decrease its risk of parasitization by *D. coccinellae* in autumn is not the migration to a distant hibernaculum site, but rather its becoming dormant early enough. The group of migrants is less affected by *D. coccinellae* because the migratory performance and the early onset of dormancy are usually combined.

#### Benefits of hibernating near breeding areas

So far, in our study we have failed to demonstrate any advantageous aspect of overwintering of *C. septempunctata* on the highly elevated peaks of the Karkonosze Mts. In contrast, it is possible to find some benefits of hibernating at lower situations. One of them, already discussed, is the lower impact of fungal pathogens. Another possible benefit of hibernation at low altitude is the early beginning of activity in spring. Individuals overwintering at lower altitudes can colonize the habitats suitable for reproduction earlier because of their earlier post-dormancy dispersal and shorter distance between the hibernaculum and breeding area. Moreover, since the ovariole maturation of many females in this group is already significantly advanced before dispersal, they can start to lay eggs quite soon after leaving their winter quarters. Honěk (1983) reported that the large *C. septempunctata* individuals, i.e. those which will be able to perform long-distance migration, are mostly produced early in the breeding season, when aphids are abundant. Later, after the crash in aphid populations, the mortality of developing coccinellid larvae is higher and the resulting adults are smaller due to partial larval starvation. Early developing progeny of the beetles overwintering near the breeding area are, thus, more likely to survive and develop into large, vital adults than the progeny of those overwintering further away, on the mountain tops. In some years, when the weather is especially unfavourable for coccinellid flight, montane hibernators may even be unable to leave their winter quarters and, consequently, to colonize breeding habitats and to reproduce (Honěk, 1989).

Why do *C. septempunctata* invest their energetic reserves in long-distance flights to and from the mountain tops? The possible causal explanation assumes the existence of hypsotactic visual orientation in pre-dormancy migration of coccinellids (Hodek, 1960; Hagen, 1962; Hodek & Honěk, 1996). Such a mechanism could evolve as an adaptation that allows insects to select dormancy sites which are dry, sunny and well protected against flooding. According to this mechanism, however, the most elevated places should strongly attract migrating beetles, even if these places are not optimal dormancy sites. Our studies seem to indicate that in the Karkonosze Mts the high summits indeed may not be optimal habitats for diapausing *C. septempunctata*. Can, thus, the hypsotactic tendency be an adaptive "blind alley" for *C. septempunctata* inhabiting mountainous regions? To try to answer this question, further comparative research in more regions is required.

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