

Accumulation of glycerol and *myo*-inositol in the overwintering nymphs of the wolf spider *Pardosa astrigera* (Araneae: Lycosidae)

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Abstract — Low-molecular-weight carbohydrates accumulated in *Pardosa astrigera* were identified and seasonal changes in the contents were correlated with the change in cold tolerance. The overwintering nymphs accumulated a high concentration of glycerol together with a small amount of *myo*-inositol. The accumulation of such molecules was at least in part associated with the acquisition of chilling tolerance but not with the depression of the supercooling point.

Key words — antifreeze agents, cryoprotectants, supercooling point, chilling tolerance

Introduction

One of the characteristics of terrestrial arthropods overwintering in the arctic or temperate regions is an accumulation of low-molecular-weight sugars and/or polyols (Storey & Storey 1991). These carbohydrates are thought to act as antifreeze agents (e.g., depressing the supercooling point (SCP) in a colligative manner) or as cryoprotectants (e.g., protecting the cell membrane and proteins at low temperatures), thereby enhancing cold tolerance (Salt 1961, Zachariassen 1985, Storey & Storey 1991, Bale 2002, Yancey 2005, Hayward et al. 2014).

To date, surveys of low-molecular-weight carbohydrates have mainly been conducted within insects (Sømme 1982). Glycerol is the most common polyol accumulated in both freeze-intolerant and freeze-tolerant insects; however, other polyols (e.g., sorbitol) or sugars (e.g., trehalose) have also been identified (Sømme 1982). Contrary to insects, only limited information is available on the low-molecular-weight carbohydrates in spiders, although the array of polyols accumulated has been examined in several species collected in winter (Kirchner & Kestler 1969; Duman 1979; Tanaka 1995). The present study aimed to provide further information on low-molecular-weight carbohydrates accumulated in overwintering spiders and their association with cold tolerance.

Pardosa astrigera, a wandering spider that commonly occurs in the grassland, is the target species in the present study. In northern Japan, the overwintering nymphs (or subadults) are found under rocks or in the litter beneath the snow cover. In the present study, we first identified low-molecular-weight carbohydrates accumulated in the

overwintering individuals and then correlated the seasonal changes in the contents with the change in cold tolerance.

Materials and methods

Spiders

Spiders used for the present study were collected from a potato field of Hokkaido National Agricultural Experimental Station (current National Agriculture and Food Research Organization, Hokkaido Agricultural Research Center), Sapporo, from August 1997 to August 1998, immediately before sugar/polyol determination and cold tolerance assessment. Although various sizes of nymphs were found from late summer to spring, only the large-sized nymphs of the last two instars were used for the present study.

Sugar and polyol contents

Spiders were homogenized individually with 4 ml of 80% ethanol in a glass homogenizer, and 1 mol erythritol was subsequently added as an internal standard according to the method of Shimada et al. (1984). The homogenate was centrifuged at $3,000 \times g$ for 15 min, and the supernatant was evaporated in a vacuum at 50°C until dryness. To the residue, 0.05 ml of trimethylsilylating reagent (TMSI-C, GL Science Inc., Tokyo) was added, and the solution was subsequently heated at 65°C for 40 min. The resulting derivative was injected into a gas chromatograph (GC-4CMPF, Shimadzu, Kyoto) with a glass column (3 mm \times 3 m) containing 1.5% OV-1. The column was heated from 130°C to 270°C at 5°C/min and subsequently maintained at the final temperature for 10 min. Compounds were identified on the basis of the retention time of standard mixtures of known

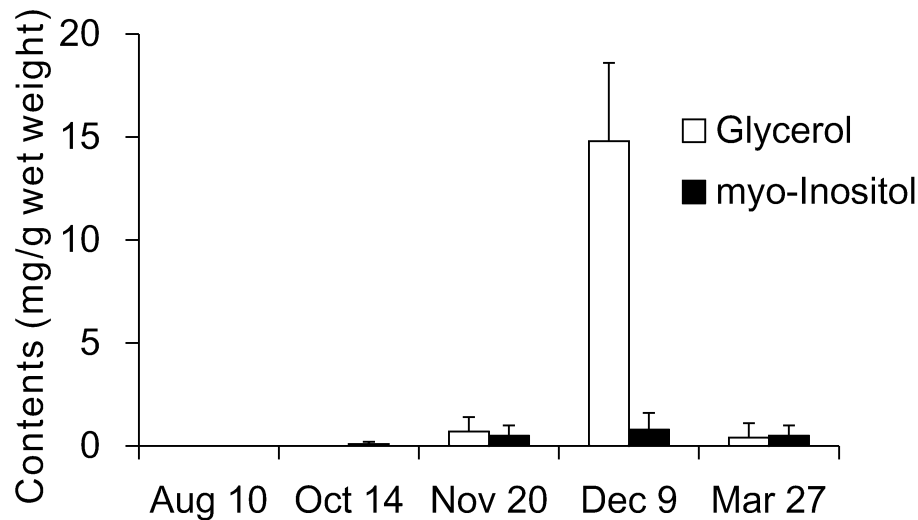


Fig. 1. Seasonal changes in glycerol and *myo*-inositol contents of *Pardosa astrigera* under natural conditions from August 1997 to March 1998. (mean \pm SD). Sample size is 4, 4, 3, 3 and 3 from August to March.

carbohydrates.

SCP

To determine SCP, spiders were individually placed into a gelatin capsule in which the specimen was in contact with the tip of a thermocouple connected to a recorder (KB681H, Rikadenki, Tokyo). The capsule was further covered by a plastic vial (4.5 cm in diameter and 8 cm in height) to reduce the cooling rate. The samples were cooled to -20°C at a constant rate of $0.05^{\circ}\text{C}/\text{min}$. The cooling rate was obtained using a program freezer, which was constructed from a deep-freezer (ESL-70A, Ebara, Tokyo), a fan heater (VF-1011, Hitachi, Tokyo), and a programmable heater controller (E5T-R91P, Omron, Kyoto). SCP was determined by a release of the latent heat due to ice formation within the body of the test animals.

Chilling tolerance

To determine chilling tolerance, spiders were individually placed into glass tubes (2 cm in diameter and 7 cm in height) that were plugged with cotton wool and subsequently buried in crushed ice in a styrene foam container. The container was placed in a refrigerator (ca. 4°C) to prevent melting of the ice. Temperatures inside the glass tube were maintained at ca. 0°C and near 100% relative humidity. Survival of spiders was checked daily by removing them from the ice and maintaining them at room temperature (25°C) for 1 h. Survival was judged by their walking activity, and only those capable of walking normally were regarded as survivors. After each assessment for survival, surviving specimens were returned to the chilled conditions described above. This entire procedure had a duration of 20 days.

Results

A chromatographic profile of the extract from the

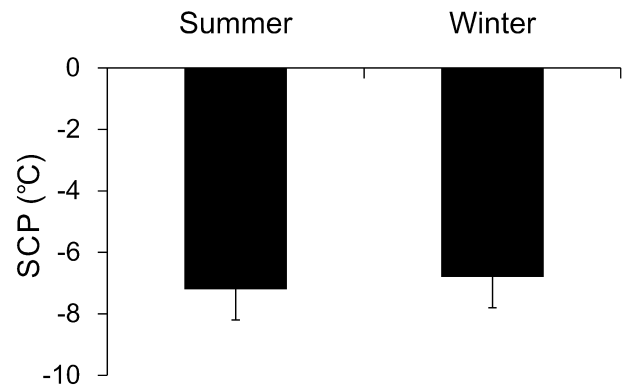


Fig. 2. Whole-body supercooling point of summer-collected (August) and winter-collected (December) nymphs of *Pardosa astrigera*. (mean \pm SD) ($N=6$ each)

nymphs of *P. astrigera* collected in December contained at least two major detectable peaks. According to the retention time, those were identified as glycerol and *myo*-inositol.

Seasonal profiles of glycerol and *myo*-inositol are shown in Fig. 1. Spiders collected in August did not accumulate any polyol. *Myo*-inositol was first detected during October, following which the concentration increased from 0.1 mg/g wet weight during October to 0.8 mg/g wet weight during December. However, from December to March, the concentration slightly decreased to 0.5 mg/g wet weight. Glycerol became detectable during November, 1 month later than *myo*-inositol, following which the concentration increased rapidly during December (14.8 mg/g wet weight). From December, the content decreased until March (0.4 mg/g wet weight). In the December samples of individual nymphs, the ratio of *myo*-inositol to glycerol was from 1:17 to 1:20. Thus, glycerol was a dominant polyol accumulated in *P. astrigera*.

Fig. 2 compares the whole-body SCP between summer-

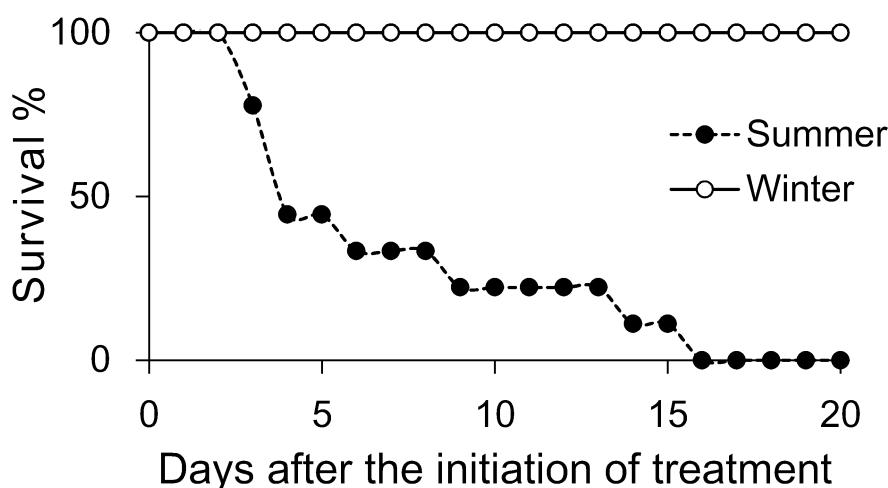


Fig. 3. Survival of summer-collected (filled circle) and winter-collected (open circle) nymphs of *Pardosa astrigera* exposed at 0°C. ($N=9$ each)

collected (August: $-8.3 \pm 0.9^\circ\text{C}$) and winter-collected (December: $-7.6 \pm 0.3^\circ\text{C}$) nymphs. No significant difference in SCP was detected (Welch's t -test: $t_6 = 1.663$, $P = 0.1446$).

Fig. 3 compares the survival of summer-collected (August) and winter-collected (December) nymphs at 0°C. Summer-collected nymphs were less tolerant to cold conditions than winter-collected nymphs. During the cold storage at 0°C, all the summer-collected nymphs died within 16 days, whereas the winter-collected nymphs did not show any mortality over the same period.

Discussion

The present study demonstrated that overwintering nymphs of *P. astrigera* mainly accumulated glycerol together with a small amount of *myo*-inositol. To our knowledge, this is only the fifth report of polyol accumulation in overwintering spiders. Kirchner and Kestler (1969) were the first to report the accumulation of glycerol in overwintering *Araneus cornutus*. In addition, the accumulation of glycerol has been reported for immature overwintering *Philodromus* and *Clubiona* spiders (Duman 1979). On the other hand, the overwintering nymphs of the house spider [*Parasteatoda* (formerly *Achaearanea*) *tepidariorum*] display a multiple polyol system of *scyllo*-inositol, *myo*-inositol, and sorbitol (Tanaka 1995). Except for the case of *P. tepidariorum*, glycerol is the only or the major polyol accumulated in spiders prior to winter. Although further confirmation is necessary, it appears that glycerol is the dominant low-molecular-weight carbohydrate present in overwintering spiders, as in other terrestrial arthropods such as insects, mites, millipedes, and scorpions (Sømme 1982).

The accumulation of low-molecular-weight carbohydrates has been associated with increased tolerance of cold conditions in several spider species (Kirchner & Kestler 1969; Duman 1979; Tanaka 1995). In *A. cornutus*, for

example, the accumulation of glycerol prior to winter approximately correlated with the depression of SCP (Kirchner & Kestler 1969), suggesting that this molecule acts as an antifreeze agent, which increases the supercooling capacity in a colligative manner. However, this is not the case in *P. astrigera*. Because a significant accumulation of glycerol and *myo*-inositol occurred from August to December (Fig. 1), no depression of SCP was observed during this period (Fig. 2).

In addition, low-molecular-weight carbohydrates accumulated during winter also function as cryoprotectants. These molecules would serve to stabilize cell membranes or protein structures both below and above freezing low temperatures, thereby enhancing the tolerance to cold conditions (Storey & Storey 1991; Bale 2002; Hayward et al. 2014). In fact, *P. astrigera* nymphs collected during December accumulated considerably more glycerol and *myo*-inositol (Fig. 1) and became more tolerant to cold conditions than those collected during August (Fig. 3). Thus, our preliminary results suggest a potential cryoprotective effect of glycerol and *myo*-inositol in *P. astrigera*, although this observation requires further confirmation.

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