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SEASONAL PATTERNS OF COLD-HARDINESS IN ANTARCTIC TERRESTRIAL ARTHROPODS

RICHARD E. LEE JR and J. G. BAUST
Department of Biology, University of Houston, Houston, TX 77004, U.S.A.

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Abstract—1. Seasonal changes in cold-hardiness of three terrestrial Antarctic arthropods were investigated.

2. Field microhabitat temperatures decreased from means of 11.2 in early February to 0.8°C by the end of March; this period represents a transition from austral mid-summer to autumn.

3. Concurrent with microhabitat cooling, two freezing susceptible species, a mite, *Alaskozetes antarcticus*, and a collembolan, *Cryptopygus antarcticus*, increased hardiness by depressing supercooling points (SCP) and accumulating cryoprotectants.

4. The freezing tolerant chironomid, *Belgica antarctica*, increased SCPs from −10.2 to −5.0°C while attaining final cryoprotectant concentrations of 11.4 μg/mg (trehalose) and 7.0 μg/mg (glucose).

INTRODUCTION

Terrestrial arthropods of temperate regions are exposed to freezing temperatures for only a few months each year. Cold-hardiness increases through the autumn to a maximum in winter and gradually declines in spring. These seasonal patterns of cold-hardening are accompanied by changes in whole body supercooling points, hemolymph melting and freezing points and cryoprotectant concentrations (Salt, 1961; Danks, 1978; Baust, 1981). In contrast, terrestrial arthropods of the sub-Antarctic may experience sub-zero temperatures at any time of the year. The amplitude of seasonal variation of average monthly air temperatures at Palmer Station (Table 1) is considerably dampened as compared to temperate regions (Baust & Edwards, 1979; Baust, 1981). Baust (1980) further documented the thermo-constancy of insect microhabitats in the Antarctic. Given this stenothermic environmental regime, it is of primary interest to determine whether (1) cold-hardiness varies seasonally in Antarctic forms or (2) natural selection has favored the retention of hardiness throughout the year. This report focuses on seasonal patterns of cold-hardiness for three terrestrial arthropods commonly found in the vicinity of Palmer Station, Anvers Island, Antarctica (64°46'S, 64°03'W).

The wingless chironomid, *Belgica antarctica* (Jacobs) is found in a variety of microhabitats including algal mats (*Prasiola crispa*), mosses (*Polytrichum* and *Bryum* spp) and animal based detritus associated with penguin rookeries and elephant seal wallows (Peckham, 1971; Baust & Edwards, 1979). This species is the only free-living holometabolous insect whose distribution extends onto the Antarctic Peninsula. The collembolan, *Cryptopygus antarcticus* Willem, and the cryptostigmatid mite, *Alaskozetes antarcticus* (Michael), have circumpolar distributions (Tilbrook, 1970; Wallwork, 1973). Both species utilize a variety of snow-free microhabitats during the austral summer.

Previous investigators have demonstrated that *Alaskozetes antarcticus* and *Cryptopygus antarcticus* are able to extend supercooling ranges to −30°C (Block *et al.*, 1978; Somme, 1978a). Further, the extent of supercooling was maximized in starved animals. Low temperature acclimation and dessication stimulated glycerol production in lab cultures of *A. antarcticus* (Young & Block, 1980). Glycerol has not been identified from *C. antarcticus* (Somme, 1978a). Larvae of *Belgica antarctica* have limited freezing tolerance during the austral summer and elaborate an array of cryoprotectants including glycerol, erythritol, trehalose and glucose (Baust & Edwards, 1979; Baust, 1980).

METHODS AND MATERIALS

Samples were collected from field sites in the vicinity of Palmer Station, Antarctica during late January–April,

Table 1. Monthly air temperatures at Palmer Station, Antarctica*

	1979								1980			
Month	M	J	J	A	S	O	N	D	J	F	M	A
Temperature (°C)												
Average	−5.0	−6.0	−7.0	−7.0	−5.0	−4.0	0.0	1.7	1.9	2.2	−1.5	−4.7
Maximum	0.0	2.0	1.0	0.0	4.0	4.0	6.0	6.0	5.0	7.0	3.0	5.0
Minimum	−13.0	−15.0	−22.0	−19.0	−17.0	−14.0	−9.0	−3.0	−2.0	−3.0	−6.0	−15.0

* (Monthly climate summary, Antarctic Journal of the United States, 1979–80).

1980. Microhabitat temperatures were measured at one cm depth in the substrate. Specimens were extracted from the substrate into ice water using Berlese–Tullgren techniques. Samples were held at ambient field temperatures until they were weighed and frozen for later cryoprotectant analysis. Supercooling point (SCP) determinations were made by attaching animals to a copper-constantan thermocouple with petroleum jelly. Specimens were cooled at a rate of 1°C/min. The SCP was recorded as the lowest temperature reached prior to the release of the latent heat of fusion (Baust & Miller, 1970). Each mean was based on 15–40 determinations.

Cryoprotectant levels were determined by high performance liquid chromatography (Waters Associates). Specimens were homogenized in distilled water (150 mg/ml) in a teflon-glass pedestal homogenizer. The homogenate was partitioned with an equal volume of chloroform: methanol (2:1 v/v), centrifuged to accelerate separation and the supernatant removed. The precipitate was washed twice with one ml of distilled water and the combined supernatants were heated 50°C for 15 min. The samples were deproteinized using 1.5 ml of 0.3 N barium hydroxide and 1.5 ml of 0.3 N zinc sulfate (10 min). The protein precipitate was pelleted by centrifugation and washed twice. The combined supernatants were evaporated to dryness at 50°C. The residue was re-suspended in 0.65 ml of distilled water, deionized with a mixed bed resin (Bio-Rad AG 501-X8), filtered (0.22 μm pore) and degassed. Carbohydrate and polyhydric alcohols were separated using a silica column modified with tetraethylenepentamine (Hendrix *et al.*, 1981). Cryoprotectant concentrations were expressed on a wet weight basis.

RESULTS

February through March represent a calendar transition from austral mid-summer to early autumn. During February (1979–80) mean air temperatures reached a yearly high (Table 1). In mid-March three days of intermittent rain were followed by a hard freeze which encased the upper 1–3 cm of substrate in ice. During this period specimens were extracted from frozen substrate. The microhabitat temperatures of the collection sites decreased steadily from February 9th (11.2°C) to the end of March (0.8°C) (Fig. 1). Thus, the sampling period included nearly the entire yearly temperature range experienced by these species.

Supercooling points of *Alaskozetes antarcticus* and *Cryptopygus antarcticus* declined concomittantly with the microhabitat temperatures (Fig. 1). Initial SCPs for both species ranged between −11.0 and −13.5°C reaching a low of −22.3°C for *C. antarcticus* and −29.2°C for *A. antarcticus* by the end of March. During the same period SCPs for *B. antarctica* increased from −10.2 to −5°C. This increase is reminiscent of similar increases in SCPs caused by the production of hemolymph nucleating agents (Zachariassen & Hammel, 1976; Somme, 1978b; Duman & Patterson, 1978; Lee *et al.*, 1981).

Cryoprotectant levels in larvae of *Belgica antarctica* are summarized in Fig. 2. Erythritol and glucose concentrations varied synchronously reaching a maximum of 6 and 9 μg/mg, respectively, in early March before dropping to comparatively low levels for the remainder of the month. Trehalose levels increased 3-fold to 11.4 μg/mg during the study period.

By the end of March substantial levels of two cryoprotectants were accumulated in *Alaskozetes antarcticus* (Fig. 3). Initially glycerol levels were less than

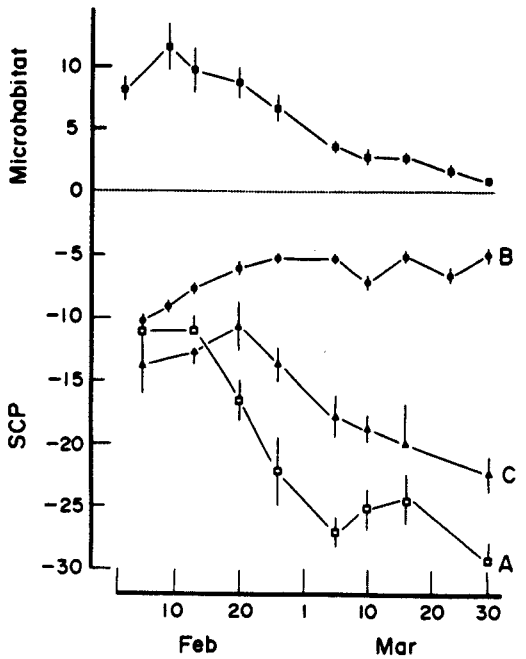

Fig. 1. Microhabitat temperatures and supercooling points ($\bar{X} \pm$ SEM) of three terrestrial Antarctic arthropods. B—*Belgica antarctica*, C—*Cryptopygus antarcticus*, A—*Alaskozetes antarcticus*.

2.5 μg/mg rising to a maximum of 23 μg/mg. Although no glucose was detected in early February, concentrations reached a maximum of 9.5 μg/mg one month later.

Few samples of *Cryptopygus antarcticus* were of sufficient size (>150 mg) to allow cryoprotectant analysis. A sample collected February 19 contained 27.7 ± 1.1 μg/mg of glucose and 2.1 ± 0.2 μg/mg of erythritol. By March 3 glucose concentration had decreased to 8.3 μg/mg with traces of erythritol and glycerol (<1.0 μg/mg).

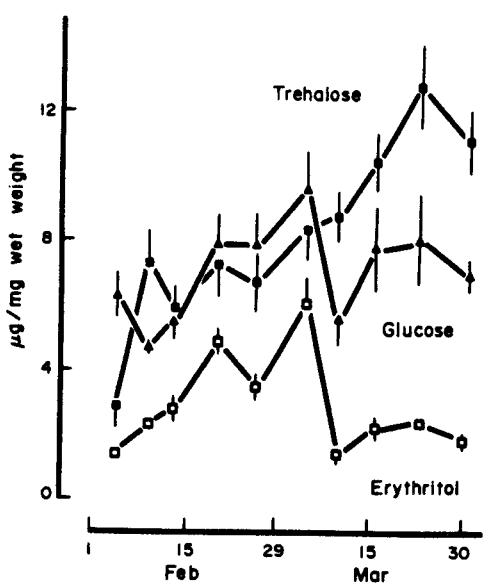

Fig. 2. Seasonal variations in whole body cryoprotectant concentrations of *Belgica antarctica* larvae ($\bar{X} \pm$ SEM).

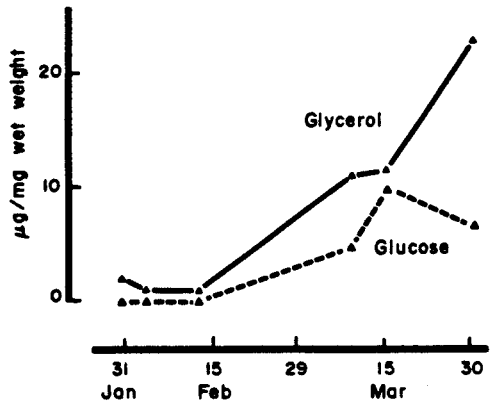

Fig. 3. Seasonal variations in whole body cryoprotectant concentrations of *Alaskozetes antarcticus*.

DISCUSSION

Based upon previous studies of insect cold-hardiness, insects may be divided into two principal classes (Baust & Lee, 1981a). Freezing susceptible species (Type I) avoid tissue freezing by extensive supercooling, while Type II species are freezing tolerant. For members of each class cold-hardening is associated with the production of high levels of polyhydric alcohols and/or low molecular weight sugars. Representatives of each category were examined in the present study; *Alaskozetes antarcticus* and *Cryptopygus antarcticus* are freezing susceptible, while *Belgica antarctica* is freezing tolerant.

Animals living in a constant environment are often characterized by limited acclimatory potential (Prosser, 1975). In light of Antarctic low temperatures and the microhabitat thermo-constancy (Baust, 1980, 1981), one might expect these species to maintain some degree of cold-hardiness throughout the year, and indeed this seems to be the case. *Belgica antarctica* is freezing tolerant and maintains an array of cryoprotective compounds throughout the summer (Baust & Edwards, 1979; Baust, 1980). Even in February, the warmest month, *Alaskozetes antarcticus* and *Cryptopygus antarcticus* maintain supercooling points in the range of -11 to -13.5°C, substantially below expected ambient temperatures. Furthermore, these two species are apparently able to rapidly enhance hardiness throughout the summer. In cultures of *A. antarcticus* and *C. antarcticus* which were maintained in the lab for 6 months, starvation at 0°C induced a marked reduction in SCP within one week (Block *et al.*, 1978). Additional lab studies with *A. antarcticus* demonstrated that low temperature acclimation increased cold tolerance independent of photoperiod (Young & Block, 1980).

Despite the maintenance of limited cold-hardiness throughout the year, this study demonstrates the seasonal enhancement of hardiness for each species during the transition from summer to autumn. The seasonal development of cold-hardiness is primarily controlled by low temperature exposure. Baust & Miller (1970, 1972) demonstrated the role of temperature in modulating glycerol, glucose and trehalose levels in the Alaskan carabid beetle, *Pterostichus brevicornis*. Specific temperature "triggers" controlling the production of sorbitol in Minnesota and Texas populations of the goldenrod gall fly, *Eurosta solidaginis*, have recently been identified (Baust & Lee, 1981a,b). Consistent with the general pattern of temperature dependent induction of hardiness, the increases observed in this study were directly correlated with a progressive reduction in microhabitat temperature.

It is apparent that mechanisms of low temperature tolerance in Antarctic terrestrial arthropods are similar to those found in temperate and arctic species. Seasonal variations in cold-hardiness are evident, however, base levels of tolerance are maintained through the austral summer.

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