Cold tolerance of two Antarctic terrestrial arthropods

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Summary. Two Antarctic arthropods, *Alaskozetes antarcticus* (Acari) and *Cryptopygus antarcticus* (Collembola) possess the ability to supercool to −30 °C, but the realisation of this potential is dependent on starvation. The mite contains glycerol in a concentration of about 1% fresh weight.

Much effort has been devoted to elucidating the mechanisms involved in the survival of subzero temperatures by Arctic[2-5] and other terrestrial invertebrates[6], but surprisingly little attention has been directed towards Antarctic animals of this kind. Apart from results of temperature preferance and tolerance experiments[7-10], there has been no reported instance of the occurrence of supercooling or the presence of cryoprotectants such as glycerol or other polyhydric alcohols in the Antarctic terrestrial invertebrate fauna. Recent work has demonstrated the ability of 2 microarthropod species (a mite and a springtail) to supercool and the presence, in the mite, of glycerol, a compound often associated with the capacity to survive exposure to low subzero temperatures[4,5].

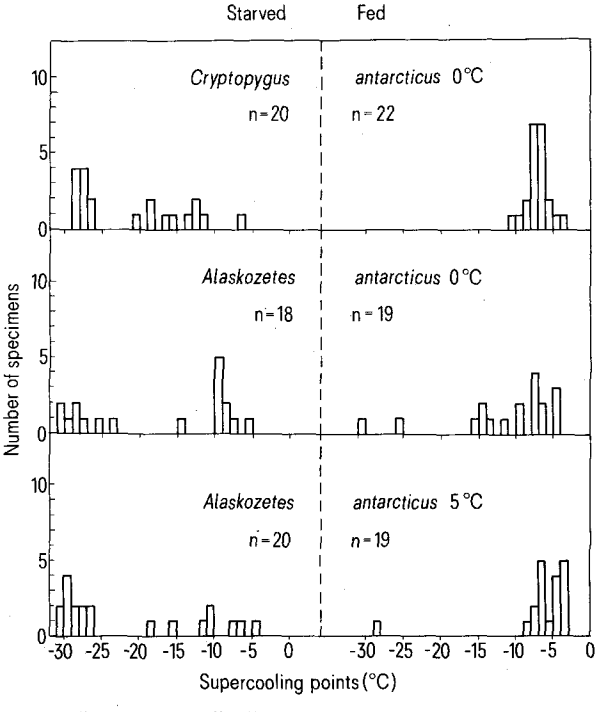
The 2 species involved in the present work are the mite *Alaskozetes antarcticus* (Michael), (Acari: Cryptostigmata) and the springtail *Cryptopygus antarcticus* Willem, (Insecta: Collembola). Both species are widespread in the maritime Antarctic and sub-Antarctic zones[11,12]. The individuals used in the experiments were collected during the austral summer of 1976–1977 near the British Antarctic Survey research station at Signy Island (60°43′S, 45°36′W), South Orkney Islands, a typical maritime Antarctic locality[13]. Here winter air temperatures may reach −25 to −39 °C and minimum temperatures within the animals' habitat are in the region of −20 to −25 °C[14]. Animals were maintained in culture at 5±2 °C from the time of their collection until they were used in the experiments (about 6 months).

Before measurements of supercooling points (lowest temperature reached before spontaneous freezing) and glycerol contents were made, *C. antarcticus* was acclimated at 0 °C, and *A. antarcticus* at 0 and 5 °C for 1 week. At each temperature 1 group of animals was fed and 1 group starved. Supercooling points were determined by the method of Salt[15], using fine copper-constantan thermocouples, for both starved and fed individuals of both species, while a paper chromatographic method[5,16] was used to test for the presence of polyhydric alchols in extracts of the animals concerned. 3 samples of each species were prepared for each acclimation temperature, using 12 individuals of *A. antarcticus* and 25 of *C. antarcticus* per sample. Use of glycerol standards on each chromatogram allowed an estimate of the concentration of unknowns to be made, since spot area is related to concentration of solution.

Results of supercooling experiments (figure) show that both species have supercooling points in the range −25 to −30 °C, which would enable them to survive winter temperatures in their habitats at Signy Island. It is also clear from the figure that the ability to supercool is strongly influenced by feeding or starvation of the animals concerned, although this is not so evident in 0 °C acclimated *A. antarcticus*. In general the results support those of Salt[15] and Sømme and Conradi-Larsen[17], who suggest that the presence of food material in the gut increases the probability of freezing occurring in a supercooled animal because such material contains efficient nucleating agents. If *A. antarcticus* had been starved for more than 1 week, a shift of more specimens to lower supercooling points would have been expected. Further experiments to this effect are being undertaken. None of the individuals used in the experiments survived the freezing process, indicating strongly that both species are freezing-susceptible and therefore depend on supercooling for survival.

The results of tests for the presence of polyhydric alcohols show that *C. antarcticus* contained no glycerol when acclimated at 0 °C, but did show the presence of another, as yet unidentified, compound on the chromatograms. *A. antarcticus*, on the other hand, contained glycerol when acclimated at 0 °C, but the substance was absent in animals maintained at 5 °C. The mean concentration (±SD) of glycerol found in the 3 samples from 0 °C was 10.1±0.35 µg mg^{-1} fresh weight (about 1%), which is a relatively low value compared to those found by Sømme and Conradi-Larsen[17] in Norwegian oribatid mites. It apparently does not affect supercooling points in this concentration, since 0 °C acclimated animals did not show lower supercooling points than those kept at 5 °C, but larger amounts may be accumulated during more prolonged storage at 0 °C or lower temperatures.

Previous work on arthropods inhabiting cold environments has suggested that there are 2 alternative ways in which such animals can survive temperatures far below the freezing point of water. 1 alternative is to avoid freezing altogether by supercooling which, apart from its ready occurrence in the absence of nucleating agents, seems to be



Supercooling point distribution histograms of *Alaskozetes antarcticus* acclimated to 0 and 5 °C, and *Cryptopygus antarcticus* acclimated to 0 °C. Number of determination is also given (n).

enhanced by the presence of glycerol[18] and other compounds[19]. The other alternative is to tolerate the extracellular freezing of the body[20], in which case the animal may produce its own nucleating agents which ensure that freezing occurs at relatively high subzero temperatures[21]. It is evident from the present data that 2 prominent and widespread members of the Antarctic terrestrial fauna have adopted the first solution, in common with mites and some insects from northern tundra environments. It is also apparent that *Nanorchestes antarcticus* Strandtmann (Acari: Prostigmata), another widely distributed Antarctic mite, has solved the problem in a similar way, since it is reported to be active at $-23\,^{\circ}C$[7].

The discovery of glycerol in extracts of *A. antarcticus* indicates another striking similarity between the strategies of north and south polar cold tolerant organisms. Research is being undertaken to clarify the role of this compound with those of starvation and acclimation to low temperatures in the development of supercooling ability.

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A homoeopathic drug controls mango fruit rot caused by *Pestalotia mangiferae* Henn.

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Summary. Effect of 1-200 potencies of ten homoeopathic drugs on the spore germination of *Pestalotia mangiferae*, the causal organism of banana fruit rot, was studied. On the basis of results of in vivo studies with inhibitory doses of drugs, Lycopodium clavatum potency 190 has been recommended for the control of the disease.

A number of methods are employed to control postharvest decay of fruits, but each method has its own limitation. In recent past some homoeopathic drugs have been shown to induce toxic effects on phytopathogens[2-6]. The present report incorporates the results of in vitro and in vivo evaluation of some homoeopathic drugs against *P. mangiferae* Henn., the causal agent of mango fruit rot.

Materials and methods. Drugs used in the study were Arsenicum album, Kali iodatum, Lycopodium clavatum, Phosphorus, Thuja occidentalis, Asvagandh, Blatta orientalis, Zincum sulphuricum, Filix mas and Kali muriaticum. The fungitoxicity of drugs was determined in terms of the inhibition of spore germination of the causal fungus. Effect of 1-200 potencies (dilutions) of each drug was studied, and the potencies were prepared in distilled water on centesimal scale as described by Khanna and Chandra[4]. To do this, one part of the mother tincture (a concentrated solution of drug) and 99 parts of distilled water were mixed in a phial by means of 10 powerful strokes. The solution was regarded as a drug having one potency and was denoted by the number 1. To make subsequent potencies, 1 part of the preceding potency and 99 parts of distilled water were mixed in a phial and were denoted with increasing potency numbers such as 2, 3, 4 ... 200. Prior to use, the drugs were sterilized by filtration through bacterial filters. Spores of the pathogens were suspended in different potencies of the drugs, and hanging drop technique of Hoffman[7] was employed to determine percentage of spore germination.

Efficacy of various homoeopathic drugs in checking mango fruit rot*

Drug	Pre-inoculation treatment		Post-inoculation treatment	
	PFI**	PRD***	PFI**	PRD***
Phosphorus potency 50	100	40.4 a	100	38.5 a
Lycopodium clavatum potency 190	3.4	2.5 b	2.8	2.0 b
Asvagandh potency 100	100	34.9 c	100	39.6 ac
Arsenicum album potency 1	100	41.3 ad	100	41.5 cd
Arsenicum album potency 89	100	35.2 ce	100	38.0 ace
Arsenicum album potency 90	100	32.6 cef	100	40.2 acdef
Zincum sulphuricum potency 1	100	38.4 acdeg	100	36.6 aeg
Zincum sulphuricum potency 2	100	40.0 adgh	100	38.9 acdefgh
Control	100	41.5 adgh	100	41.8 cdfh
C.D. at 5%		3.58		2.93

* Results were statistically analyzed for analysis of variance and Duncan's Multiple Range Test at 5% level. Numbers followed by the same letter are not significantly different within columns. ** PFI, percentage fruit infected; *** PRD, percentage rot developed.