

1.20 COLD HARDINESS IN THE IXODID TICKS (IXODIDAE)

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Researchers have suggested that low temperatures in winter limit the geographical range of various tick species (Sonenshine 1979; Needham and Teel 1991). Several field studies have examined the impact of chilling and low temperatures on various ixodid species (*i.e.* Sonenshine 1979 and references therein, Koch 1984). While these types of observational studies are valuable in examining overwintering mortality, they often do not explain what specific factors (*e.g.* freeze injury, ice nucleation) associated with overwintering are most limiting and therefore have the greatest impact on distribution of the species. Laboratory studies, while more removed from the field conditions, allow for controlled and specific investigation of physiological mechanisms for surviving cold stress. These types of studies on tick cold-hardiness are particularly lacking. Our purpose is to review the laboratory data that are available on cold tolerance in ixodids and to relate known mechanisms of cold-hardiness in insects with microhabitat conditions of overwintering ticks.

MATERIALS AND METHODS

General survival strategies exhibited by arthropods that naturally experience subzero temperatures can be categorized as: 1) behavioral avoidance of exposure to extreme temperatures; 2) avoidance of internal ice formation by promoting supercooling of body fluids; and 3) toleration of freezing of body fluids (see reviews in Lee and Denlinger 1991). Behavioral avoidance of temperature extremes can be used to enhance the viability of a freeze avoidance strategy.

The supercooling point (*e.g.*, the temperature at which ice forms in an organism) is determined by chilling the animals at a slow constant rate (*ca.* 1° C/min) while monitoring its body temperature using an external thermocouple. The lowest temperature prior to a transient rise, indicating heat released during ice formation, is considered the supercooling point. Because small volumes of water are more likely to supercool than larger

volumes (Lee 1991), supercooling is a more viable alternative for small organisms. Organisms can further enhance their supercooling by purging themselves of internal ice nucleators, and/or by avoiding inoculative freezing due to contact with external ice.

There are two ways freeze susceptible animals are killed by cold (Lee 1991). Some, which tolerate chilling down to temperatures immediately above their supercooling point, are killed when ice crystals form in their tissues. This pathology is referred to as freeze injury. Other organisms are killed by subzero temperatures well above their supercooling points, which is termed chill injury. For these animals, the supercooling point is physiologically irrelevant.

Lower lethal temperature (LLT) can be assessed experimentally in two ways. Organisms can be kept at a constant low temperature for a variety of times to determine the longest time interval at which a significant proportion of individuals survived. One can also keep the time constant and expose the animals to a variety of temperatures. Unfortunately, there is no generally accepted standard, and data comparisons using these different approaches is problematical. However, as previously mentioned, for freeze susceptible species supercooling point determinations are practically meaningless unless the LLT is also assessed.

Among the insects, increased cold tolerance is commonly observed in response to low temperature acclimation or as a part of entry into diapause. In freeze intolerant insects, this conditioning may include removal or inactivation of endogenous ice nucleators, production of thermal hysteresis (*i.e.*, "antifreeze") proteins, and accumulation of low molecular weight cryoprotectants, particularly glycerol (see reviews in Lee and Denlinger 1991).

Increased amounts of glycerol and other low molecular weight cryoprotectants may be due in part to increased glycogen phosphorylase activity in the 0 to 5° C range, thereby increasing glycolytic intermediate levels (Storey and Storey 1992). Glycerol serves as a colligative antifreeze, and lowers the supercooling and

freezing points of an animal, although high concentrations (up to 5 M) are required for this purpose. Elevated concentrations of glycerol and other low molecular weight cryoprotectants are also found in freeze intolerant insects, and are sometimes associated with significant decreases in lower lethal temperatures. These observations indicate that glycerol protects against chill injury as well as damage from freezing. While a high glycerol concentration is required to significantly reduce the temperature of ice formation, it is not clear how much is required to increase chill tolerance.

RESULTS AND DISCUSSION

The supercooling point (SCP) for unengorged nymphs and adults of *Amblyomma americanum* was $-20.9 \pm 0.9^\circ\text{C}$ and $-15.6 \pm 2.5^\circ\text{C}$ respectively, the SCP of nymphal and adult *Dermacentor variabilis* was $-22.1 \pm 2.4^\circ\text{C}$ and $-19.3 \pm 1.8^\circ\text{C}$ respectively, (unpublished data).

The SCP for *D. marginatus* (Sulzer) and *D. reticulatus* Fabricus adults ranged between -16 and -22°C . The SCP for *Haemaphysalis leachi* (Audouin) adults, *Hyalomma lusitanicum* Koch adults, *Rhipicephalus appendiculatus* Neumann adults and *R. turanicus* Pomerantser adults was -18°C . The SCP of *Ixodes ricinus* L. adults and engorged nymphs of *R. sanguineus* (Latreille) was -11 and -22°C respectively (Dautel and Knülle, 1994). The SCP of *I. scapularis* Say unengorged nymphs was -21.7°C (unpublished data) and the SCP of *I. uriae* White ranged between -11 and -20°C (Lee and Baust, 1987). Although the lower lethal temperature (LLT) for most species and stages was relatively low, generally $< -10^\circ\text{C}$, it was still distinctly above their respective supercooling points. The LLT temperature has only been investigated in four species of ticks *A. americanum* (nymphs and adults), *A. cajennense* (Fabricus) (adults), *D. variabilis* (nymphs and adults), and *I. scapularis* (nymphs). The LLT of these ticks are $2\text{h}@-12$ and $2\text{h}@-12.5^\circ\text{C}$ for *A. americanum* nymphs and adults respectively, $1.5\text{h}@-12.5^\circ\text{C}$ for *A. cajennense* (unpublished data P. D. Teel, O. S. Strey, M. T. Longnecker, and G. R. Needham), $2\text{h}@-14^\circ\text{C}$ for *D. variabilis* nymphs and adults, and $2\text{h}@-10^\circ\text{C}$ for *I. scapularis* (unpublished data). These data demonstrate that cold-induced mortality in these ticks is due to chill injury rather than freeze injury. Similarly, freeze tolerance has not been reported in ticks and has only been found in one mite species in more than a dozen examined (Somme 1981, Schmid 1986).

How do the observed LLTs relate to temperatures naturally experienced by overwintering ticks? In a Southwest Ohio locale for example, continuous temper-

ature recordings from the leaf litter during the winter of 1994 indicated continuous sub-zero temperatures for up to 41 days, but these readings were generally above -4°C , and never lower than -5°C (Burks, unpublished data). In Minnesota, soil surface temperatures in a similar habitat ranged from -2°C down to -6°C between December, 1980 and January, 1981 (Schmid 1986). And the habitat of the Antarctic tick, *Ixodes uriae*, was also reported to maintain a temperature in the range of 0°C to -2°C (Lee and Baust 1981).

In contrast to insects, there is little indication of increased cold tolerance in ticks conditioned by cold acclimation or short photoperiods. A slight increase in chill tolerance was observed for *A. americanum* nymphs conditioned by four weeks of exposure to 4°C and a 10:14 L:D photoperiod (Burks, unpublished data). These conditions are similar to those that induce diapause in this stage and species (Pound and George, 1988). Interestingly, a slight but significant increase in osmolarity (ca. 20 mOsm) was also detected for *A. americanum* adults in response to 4°C exposure for only 2 h (Needham, unpublished data). If this increase in osmolarity is due to glycerol production, a sufficient amount may not have been produced to enhance cold-hardiness since survival was not enhanced, this was suggested by Lee and Baust (1987).

SUMMARY

We summarized methods of insect cold tolerance, and physiological mechanisms by which chill tolerance is enhanced in insects, and contrasted these mechanisms with the data available. Ticks avoid the coldest extremes of their climate by seeking a sheltered (off-host) microhabitat. They generally have low supercooling points as a consequence of their small size and the apparent absence of efficient internal ice nucleators. The lower lethal limits for those species examined are high compared to their supercooling point, indicating that chilling causes mortality rather than internal ice formation. However, the lower lethal temperatures are distinctly lower than temperatures actually recorded in tick microhabitats during the winter. In contrast to many insects, there is little evidence of increased chill tolerance by ixodids examined thus far in response to conditioning by mild chilling or short photoperiod. We speculate that cold-induced mortality results from contact with external ice nucleators, or that prolonged exposure to moderate temperatures may challenge ticks.

Future cold-hardiness studies on ixodids should include frequent temperature recordings, (e.g. hourly intervals), from the species microhabitat. To determine

if temperature limits the geographical range of species or species strains, microhabitat temperatures must be mimicked in laboratory situations and ticks must be exposed to these temperatures holding other variables (*e.g.* humidity) constant. The role of cold conditioning should be examined further as well. Temperature responses of key glycolytic enzymes should be compared for temperate tick species that do not exhibit cold conditioning with arthropods that do.

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