Surviving winter in a frozen state: the search for ice-binding proteins in a freeze tolerant insect

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While the function of ice-binding proteins (IBPs) has been reasonably established in freeze sensitive animals, they are considerably less understood in those that are freeze tolerant. These organisms permit the accumulation of extracellular ice while still maintaining an intracellular liquid state. The larvae of the goldenrod gall fly, *Eurosta solidaginis*, is a well-characterized freeze-tolerant insect that overwinters in galls located on the stems of the annual goldenrod plant. Able to endure the freezing of up to 65% of their total body water, these larvae use a variety of different mechanisms to survive temperatures as low as -30 °C. It is currently unknown if these larvae produce IBPs; however, since IBPs are known to play important roles in other freeze tolerant organisms, it is possible that they could be found in this species. To investigate the possible roles of IBPs in a freeze tolerant insect, *E. solidaginis* larvae acclimated to three different temperatures representing the autumn to winter periods were carefully examined for ice-associating properties using INA, IR inhibition and TH/ice morphology assays. Specimens collected during the early autumn did display evidence of ice recrystallization inhibition and ice shaping activity. These activities were not seen in late autumn and winter samples; however, larvae acclimated to winter temperatures did show type III ice nucleation.

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