

Unveiling winter survival strategies: physiological and metabolic responses to cold stress of *Monochamus saltuarius* larvae during overwintering

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Abstract: (1) Background: *Monochamus saltuarius* is a destructive trunk-borer of pine forest and an effective dispersal vector for pinewood nematode (PWN), a causative agent of pine wilt disease (PWD), leading to major environmental disasters. Cold winter temperatures determine insect survival and distribution. Climate change is projected to shift insect vector range borders northward, increasing PWD risk in high-latitude and high-altitude regions. However, little is known about the cold tolerance of *M. saltuarius*, making it difficult to assess their potential risk range. **(2) Results:** We proved that dead *Pinus koraiensis* trunks provide no insulation to larvae. The *M. saltuarius* larvae are freeze-tolerant species. Unlike most other freeze-tolerant insects, they can actively freeze extracellular fluid at higher subzero temperatures by increasing their supercooling points. The main energy sources for larvae overwintering are glycogen, mid-late switch to lipid. The water balance showed a decrease in free and an increase in bound water of small magnitude. Cold stress promoted lipid peroxidation, thus activating the antioxidant system to prevent cold-induced oxidative damage. We identified eight core pathways associated with cold stress as well as 39 key metabolites, including seven cryoprotectants: maltose, UDP-glucose, D-fructose 6P, galactinol, dulcitol, inositol, and sorbitol. Cysteine and methionine metabolism and alanine, aspartate and glutamate metabolism are the most significant pathways linked to antioxidation and energy production. **(3) Conclusions:** Our findings have important implications for modeling population dynamics, predicting potential suitable areas and assessing the risk of this pest, thereby contributing to assessing the risk of PWN and controlling the further spread of PWD. **Keywords:** Cold stress, overwintering strategies, energy sources, antioxidant activity, metabolomics, pathway-metabolite net