Frost hardiness of ectomycorrhizal fungi in pure culture

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Introduction

Although soil temperatures tend to be less extreme than air temperatures, soil frost is common in the boreal zone. Aboveground parts of northern trees are often adapted to tolerate temperatures down to -80˚C, but in some studies, fine roots have not tolerated lower temperatures than -5˚C. On the other hand, in the field, massive diebacks of mycorrhizas have not been reported in winter even near the soil surface. We hypothesised that 1) mycorrhizal fungi tolerate below-zero temperatures, and that 2) hydrophobic species are more tolerant than hydrophilic ones, due to the relation between the occurrence of free water and the initiation of ice crystallisation.

Material and methods

Pure cultures of *Laccaria laccata*, *Hebeloma* sp. and *Suillus luteus* were grown in liquid medium at room temperature (23˚C) for 5 weeks. Subsequently, intact pieces of mycelium were rinsed with deionised water, drained, and placed in test tubes. Different groups of samples were subjected to different temperatures between +5˚C and - 48˚C for 4 h, with a cooling and warming rate 5˚C h-1. Relative electrolyte leakage (REL) was used for viability assessment. For an estimation of the lethal temperature for 50% of the samples (LT50), a sigmoid function was fitted to the REL data. In a second experiment, the survival of samples of the same isolates and additionally *Suillus variegatus* was assessed by aseptically transferring them to Hagem agar plates after frost exposure, and recording their survival as growth visible without magnification.

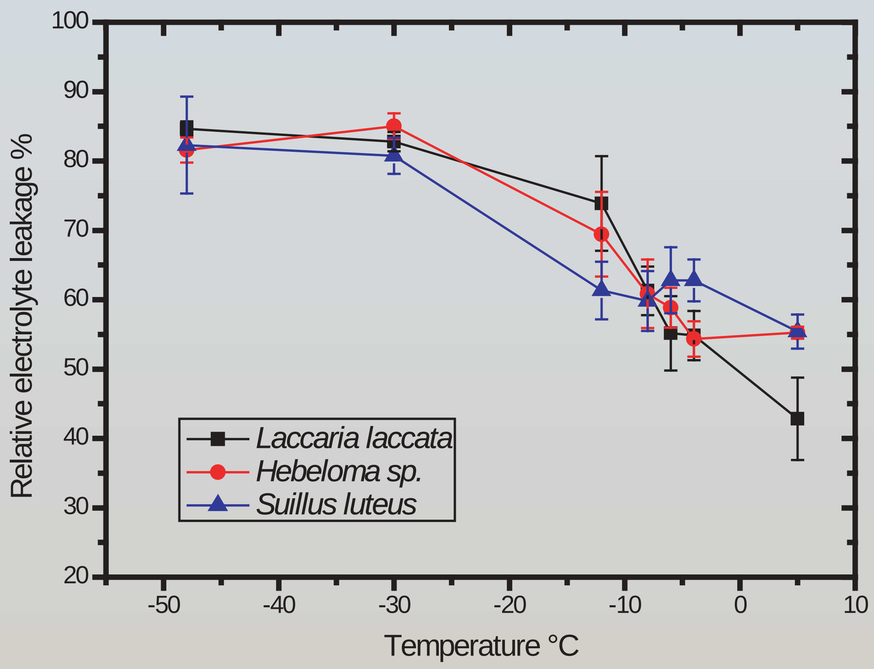

Figure 1. Relative electrolyte leakage % from samples exposed to different temperatures.

Results

The estimated LT50 by REL was -8.3 for *Suillus luteus*, -11.5 for *Laccaria laccata*, and -13.5˚C for *Hebeloma* sp. (Figure 1). All isolates resumed growth after exposure to -8˚C, and at -12˚C, the survival rate of *Hebeloma* and *S.variegatus* was still 100% (Table 1). Furthermore, part of the specimens resumed growth even after exposure to -48˚C. In the lowest temperatures, there was a delay in the start of the growth, as part of the isolates that showed growth in week 4, had not grown in the first week.

Discussion

The fungi grown at room temperature tolerated lower temperatures than expected. Possible explanations to the hardiness are delayed ice crystal formation by supercooling, and tolerance to apoplastic freezing. Both may be connected to cell wall properties which may prevent the initiation of ice nucleation. However, there was no evidence of better cold hardiness in the hydrophobic *Suillus* species.

The following step is to study the frost hardiness of different mycorrhizas. These studies can yield insights into the reasons for the distribution of different mycorrhizas in different climatic zones, and into the changes that global warming may cause in the fitness of plants, fungi, and their symbioses.

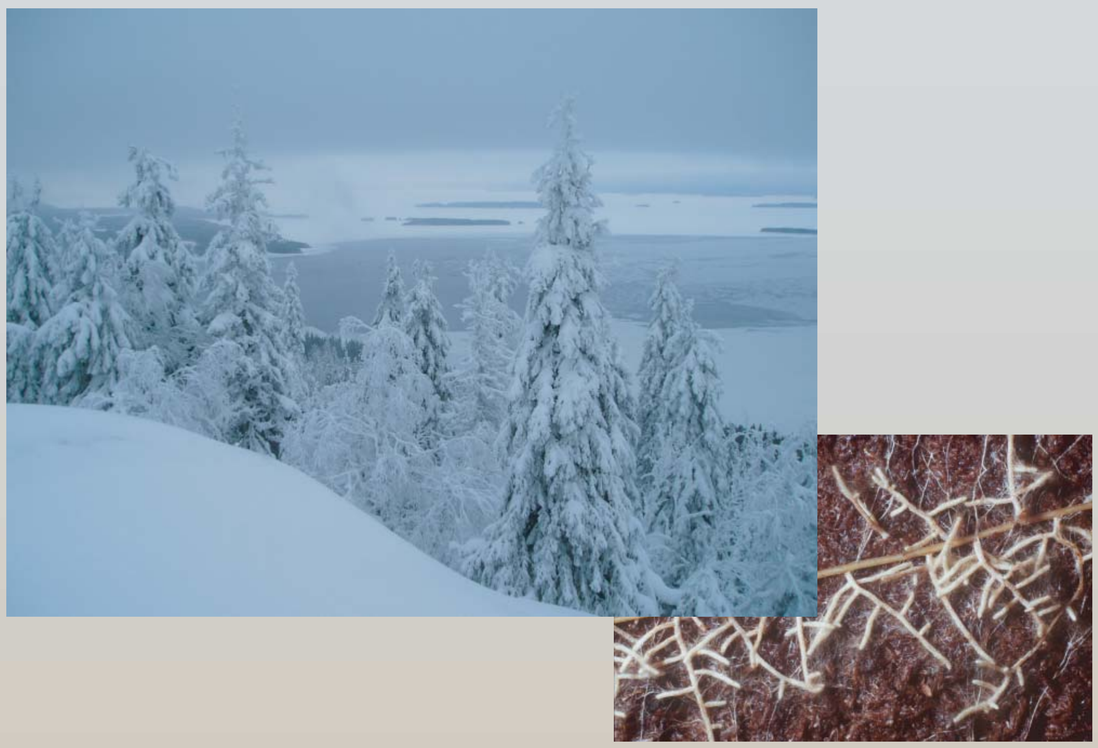

Table 1. Percent of samples showing growth one and four weeks after a cold exposure treatment.

	Laccaria laccata		*Hebeloma sp.*		*Suillus luteus*		*Suillus variegatus*	
T˚C	Week 1	Week 4	Week 1	Week 4	Week1	Week 4	Week 1	Week 4
5	100	100	100	100	100	100	100	100
-4	100	100	90	100	95	100	100	100
-8	100	100	100	100	100	100	100	100
-12	83	83	100	100	71	86	58	100
-30	29	57	17	67	0	7	20	60
-48	18	64	0	0	0	0	11	11