From Forest Nursery Notes, Winter 2011

First frost: Effects of single and repeated freezing events on acclimation in *Picea abies* and other boreal and temperate conifers

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**Article Info**

Article history:
Received 15 November 2009
Received in revised form 15 January 2010
Accepted 15 January 2010

Keywords:
Conifer
Frost
Freezing
Cold
Tolerance
Hardiness
Acclimation

**Abstract**

In experiments with needles of *Picea abies*, we tested the specific hypothesis that a single night of freezing acts as a signal that triggers a rapid increase in low temperature (LT) tolerance, and the more general hypothesis that repeated or prolonged freezing stimulates increased LT acclimation. In three growth chamber experiments involving acclimation under early- to mid-autumn light and temperature conditions followed by one or more freezing treatments, we found no significant effect of a single night of freezing on LT tolerance, and only limited and inconsistent effects of repeated and prolonged freezing. We also tested the effect of prolonged storage at −5 °C on LT tolerance on samples of three boreal and three temperate conifer species during acclimation under field conditions, and again found no consistent enhancement of LT tolerance attributable to freezing in either group. In agreement with our own and others’ anecdotal observations that some species can attain nearly maximal LT tolerance in the absence of freezing under field conditions, we conclude that freezing is neither required nor a major influence in LT acclimation, at least in well-studied boreal conifer species, while the effects of freezing on temperate conifers are not as well-documented. We conclude that freezing treatment of conifer seedlings to ensure sufficient hardiness for late planting seems to offer little practical advantage.

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1. Introduction

In temperate and boreal environments, woody plant acclimation to low temperature (LT) begins in late summer in response to decreasing day length, coincident with the development of dormancy in buds (Bigras et al., 2001; Li et al., 2004). Studies of acclimation in a variety of woody plants show that exposure to chilling (0–10 °C) or freezing (below 0 °C) temperatures initiate further acclimation, with some suggesting that freezing temperatures are required for some species to acquire maximum LT tolerance (Sakai, 1966; some early studies of deciduous species reviewed in Weiser, 1970; studies of acclimation in conifers reviewed in Bigras et al., 2001; Beck et al., 2004; Søgaard et al., 2009). Consideration of the biophysics of freezing in plants suggests that an early frost event could act as an unambiguous signal of impending winter. When the environmental temperature falls below 0 °C, water in the plant may supercool by a few degrees, but with sufficiently low temperature (−2 to −5 °C) freezing will begin in the xylem or other extracellular regions, possibly triggered by nucleators (Lee and Hammel, 1982). This results in dehydration of the unfrozen cells as extracellular ice masses grow by drawing water from the unfrozen cytoplasm. Simple calculations using the equation for melting point depression show that a 2 osmolar solution, a typical intracellular value for a conifer during acclimation (Tyree et al., 1978), will lose about 25% of its water in equilibrium with ice at −5 °C, a typical temperature for a "hard" frost event occurring during the autumn months and sufficient to trigger extracellular freezing in plants. Thus, extracellular freezing during a night of frost will result in rapid dehydration of the cells. This could act as a warning of further freezing to come, to which the plant could then respond by initiating the final stages of LT acclimation. These considerations give rise to the specific hypothesis, here called the first frost hypothesis, that a single occurrence of extracellular freezing triggers a rapid phase of acclimation that ensures that the plant is ready to tolerate more extreme freezing events. Here we report on the results of some experiments with *Picea abies* (L.) Karsten aimed at testing this hypothesis, and additional treatments and experiments to assess the effects of repeated freezing events on LT acclimation in needles of *P. abies* and other conifer species from both temperate and boreal regions.

We also examined the effects of prolonged, continuous freezing on LT tolerance in conifers. Sakai (1966) reported that frozen storage at −3 to −5 °C dramatically improved LT tolerance in boreal *Salix, Populus*, and *Larix* twigs and buds. To test for similar effects in conifer foliage, with potential application in improving LT