



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

G. Richard Strimbeck^{a,*}, Trygve D. Kjellsen^b

^a Department of Biology, Norwegian University of Science and Technology, Realfagbygget, Høgskoleringen 5, 7491 Trondheim, Norway

^b StatoilHydro Research Centre, 7005 Trondheim, Norway

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 $-5\text{ }^{\circ}\text{C}$ 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.

© 2010 Elsevier B.V. All rights reserved.

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 ($0\text{--}10\text{ }^{\circ}\text{C}$) or freezing (below $0\text{ }^{\circ}\text{C}$) 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; Sogaard 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 $0\text{ }^{\circ}\text{C}$, water in the plant may supercool by a few degrees, but with sufficiently low temperature (-2 to $-5\text{ }^{\circ}\text{C}$) 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 $-5\text{ }^{\circ}\text{C}$, 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 $-5\text{ }^{\circ}\text{C}$ 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

* Corresponding author. Tel.: +47 73551284; fax: +47 73596100.
E-mail addresses: richard.strimbeck@bio.ntnu.no (G.R. Strimbeck),
TDKJ@statoilhydro.com (T.D. Kjellsen).

tolerance for late planting, we assessed changes in LT tolerance after dark storage at $-5\text{ }^{\circ}\text{C}$ for periods of 2–4 weeks in adult trees of boreal and temperate *Abies*, *Picea*, and *Pinus* species, growing in an arboretum in a relatively mild winter climate in Trondheim, Norway. This experiment was an extension of our monitoring of LT tolerance during a complete acclimation–deacclimation cycle, as reported in a previous paper (Strimbeck et al., 2008).

2. Materials and methods

2.1. Growth chamber experiments

In December 2004, 2-year-old dormant nursery-grown *P. abies* seedlings of a local, low-elevation seed source (Skogplanter Midt-Norge AS, Skjerdingsstad Nursery, Kvål, Norway, www.spmn.no; K2 seed source, Sør-Trøndelag province, 150–249 m elevation, lifted and stored at $-2\text{ }^{\circ}\text{C}$ in October, 2004) were planted in 1.5 l pots in a 1:1 mix of potting soil and perlite. These were grown in a greenhouse at $20\text{ }^{\circ}\text{C}$, under quartz halogen lights at a minimum $200\text{ }\mu\text{mol m}^{-2}\text{ s}^{-1}$ PAR and 20/4 h D/N, augmented by natural light during the daytime hours, and watered three times a week with no additional fertilization for at least 7 weeks before beginning LT acclimation treatments. Programmable environmental chambers (Vötsch Industrietechnik GmbH, Balingen-Frommen, FRG; <http://www.v-it.com/en/vit/start>) were used to impose photoperiod and temperature treatments, without replication for chamber effects. In all experiments, light was increased and decreased linearly from darkness to $200\text{ }\mu\text{mol m}^{-2}\text{ s}^{-1}$ PAR over the first and last hour of the day period, respectively, and maintained at the same value through the remainder of the daylight period. Humidity was maintained at or above 60%. Where possible, extra plants were kept in the growth chambers as edge and reserve plants.

For freezing treatments, plants were removed from the growth chamber after the end of the daylight period and transferred to a programmable freezing chamber for a freeze/thaw cycle, then returned to the growth chamber before the beginning of the next light period. Plants were transferred in 5 cm thick styrene foam boxes to minimize environmental effects during transfer. We used a minimum temperature of $-6\text{ }^{\circ}\text{C}$ to ensure extracellular nucleation and substantial freeze dehydration of the needles, as is assumed in the first frost hypothesis. Although the freezing treatments were not applied independently for individual plants within treatment groups, freezer conditions were closely controlled and monitored and we observed no significant departures from the programmed temperature in any of the experimental freezing treatments, so for the purpose of analysis we treated individual seedlings as experimental replicates.

After treatment, all the current-year growth (produced during growth in the greenhouse) of each plant was harvested for LT tolerance assessment. Plants were returned to growth chambers after harvest to maintain normal chamber conditions, and discarded at the end of each experiment. Relative LT tolerance was estimated by controlled freezing of needle sections measurement of relative electrolyte leakage (REL), and nonlinear curve fitting following previously published methods (Strimbeck et al., 2007). We prepared a homogeneous sample of 5 mm needle sections from all the current-year shoots from each plant, and cooled ca. 0.1 g subsamples to each of 10–16 test temperatures on each date, with samples held at $4\text{ }^{\circ}\text{C}$ as the maximum temperature and minimum temperatures ranging from -30 to $-60\text{ }^{\circ}\text{C}$ depending on the anticipated level of LT tolerance of the tissue. This approach allowed us to evaluate individual LT tolerance at the time of harvest in each plant used in the experiment. T_m , the midpoint of a sigmoid curve fit to REL data for each plant, corresponds to LT_{50} , the temperature resulting in 50% lethal injury, and was used as the response variable in analyses.

Table 1

Treatment details and results of freezing experiments on low temperature tolerance of *Picea abies* needles. Values are the mean of eight (Experiments 1 and 2) or six (Experiment 3) 2-year-old plants in each treatment group. T_m is the midpoint of a sigmoid curve fitted to electrolyte leakage data for each plant, equivalent to LT_{50} when freezing results in complete mortality, as is the case in all of these experiments.

Treatment	T_m , $^{\circ}\text{C}$	HSD test ^a
<i>Experiment 1</i> (12/12 h D/N)		
20/15 $^{\circ}\text{C}$ D/N, no frost	-9.3	a
20/15 $^{\circ}\text{C}$ D/N, 1 night frost	-10.4	ab
15/5 $^{\circ}\text{C}$ D/N, no frost	-12.6	b
15/5 $^{\circ}\text{C}$ D/N, 1 night frost	-12.2	b
<i>Experiment 2</i> (8/16 h, 15/5 $^{\circ}\text{C}$ D/N)		
No frost, day 9	-14.2	a
1 night frost, day 9 ^b	-13.0	a
2 nights frost, day 9	-17.0	ab
9 nights frost, day 9	-21.4	b
No frost, day 16	-13.6	a
1 night frost, day 16 ^b	-15.4	ab
2 nights frost, day 16	-14.1	a
16 nights frost, day 16	-18.4	ab
<i>Experiment 3</i> (8/16 h 10/2 $^{\circ}\text{C}$ D/N)		
No frost	-34.7	a
1 night frost	-37.6	a
7 nights frost	-34.7	a
7 days continuous frost	-39.3	a

^a Within each experiment, means with the same letter are not significantly different at $\alpha=0.05$.

^b Frost treatment on day 7, T_m determined 2 and 9 days after treatment.

We conducted three different acclimation experiments involving different combinations of photoperiod, acclimation temperature, and freezing treatment (Table 1).

Experiment 1: This experiment was designed to test the first frost hypothesis, which predicts that a single early autumn frost event will trigger accelerated LT acclimation. After 7 weeks growth in the greenhouse, 60 plants were transferred to a growth chamber for short day-high temperature (SDHT; 12/12 h and 20/15 $^{\circ}\text{C}$ D/N) treatment. After 3 weeks in these conditions, half the plants were transferred to a second chamber for short day-low temperature (SDLT; 12/12 h and 15/5 $^{\circ}\text{C}$ D/N) treatment. These day length and temperature treatments we selected to approximate local conditions at autumnal equinox under warm to cool conditions. Four weeks after the start of SDLT treatment (14 weeks after planting), 8 SDHT and 8 SDLT plants were transferred to the freezer for a single night of frost, then returned to the growth chambers. For night frost, plants were transferred to the freezer at the end of the day period, held at $0\text{ }^{\circ}\text{C}$ for 1 h, cooled to $-6\text{ }^{\circ}\text{C}$ over 2 h ($3\text{ }^{\circ}\text{C h}^{-1}$), held at $-6\text{ }^{\circ}\text{C}$ for 2 h, warmed to $0\text{ }^{\circ}\text{C}$ over 2 h, and held at $0\text{ }^{\circ}\text{C}$ for 1 h before transfer back to the growth chambers. Unfrozen controls were kept in both growth chambers. Plants were randomly assigned to treatment groups. Frost tolerance was assessed 7 days after night frost treatment in 7 plants from each treatment group. The results were analyzed as a 2×2 factorial with pretreatment temperature and frost treatment as the main effects and T_m as the response variable. The four treatment group means were compared *a posteriori* using Tukey's HSD.

Experiment 2: This experiment was designed to compare the effects of single and repeated freezing events on LT acclimation in mid-autumn conditions. Plants were potted at the same time as those in the first experiment, and kept in the greenhouse until after completion of the first experiment. After 17 weeks growth in the greenhouse, 70 plants were transferred to a growth chamber under 8/16 h and 15/5 $^{\circ}\text{C}$ D/N. The 8-h photoperiod corresponds approximately to a calendar date of 1 November at the local latitude. After 4 weeks under these conditions, randomly assigned subsets of plants were exposed to four frost treatments: (1) no

Download English Version:

<https://daneshyari.com/en/article/88501>

Download Persian Version:

<https://daneshyari.com/article/88501>

[Daneshyari.com](https://daneshyari.com)