Silva Fennica 1990, Vol. 24 N:o 4: 335–340

# Frost hardiness of Scots pine seedlings during dormancy

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*TIIVISTELMÄ: MÄNNYN TAIMIEN PAKKASKESTÄVYYS LEPOVAIHEEN AIKANA*

Valkonen, M-L., Hänninen, H., Pelkonen, P. & Repo, T. 1990. Frost hardiness of Scots pine seedlings during dormancy. Tiivistelmä: Männyn taimien pakkaskestävyys lepovaiheen aikana. Silva Fennica 24(4):335–340.

The relationships between bud dormancy and frost hardiness were examined using two-year-old *Pinus sylvestris* L. seedlings. The chilling temperatures used were +4 and –2 °C. To examine the dormancy release of the seedlings, a forcing technique was used. Frost hardiness was determined by artificial freezing treatments and measurements of electrical impedance. At the start of the experiment, the frost hardiness of the seedlings was about –25 °C. After rest break, the seedlings kept at +4 °C dehardened until after eight weeks their frost hardiness reached –5 °C. At the lower chilling temperature (–2 °C) the frost hardiness remained at the original level. When moved from +4 to –2 °C, seedlings were able to reharden only after the time required for bud burst in the forcing conditions had reached the minimum.

Männyn 2-vuotiaiden taimien lepovaiheen ja pakkaskestävyyden välistä yhteyttä tutkittiin erilaisten kylmäkäsittelyiden avulla. Kahden taimiryhmän käsittelylämpötilat olivat +4 ja –2 °C. Näiden lisäksi siirrettiin ryhmä taimia kahden viikon välein +4 asteen lämpötilasta –2 asteen lämpötilaan. Lepovaiheen päättymistä tutkittiin hyötökokeen avulla. Pakkaskestävyys määritettiin keinotekoisen pakkaskäsittelyn ja impedanssimittauksen avulla. Kokeen alussa taimien pakkaskestävyys oli noin –25 °C. Silmulevon purkautumisen jälkeen +4 °C:n kylmäkäsittelylämpötilassa käsitellyt taimet suveentuivat aina –5 °C:n pakkaskestävyystasolle saakka. Alemmassa kylmäkäsittelylämpötilassa (–2 °C) taimien pakkaskestävyydessä ei tapahtunut merkittäviä muutoksia. Lämpötilan aleneminen +4 asteesta –2 asteeseen aiheutti uudelleenkaraistumista vasta, kun kasvun alkamiseen hyötöolosuhteissa kuluva aika oli saavuttanut miniminsä.

Keywords: annual cycle, chilling requirement, *Pinus sylvestris*, rest, cold hardiness.
ODC 161+181.5

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Accepted January 25, 1991

# 1. Introduction

Trees of cool and temperate regions are able to survive despite great seasonal variation in environmental conditions. The annual rhythm of such trees, a timetable according to which specific stages of development are achieved, is synchronized with the changing environment. In late summer and autumn, growth ceases and trees begin to develop tolerance to environmental conditions that are unfavourable for growth.

During the rest period, which follows growth cessation, internal factors in the trees prevent growth regardless of the prevailing environmental conditions. Rest is broken when the growth competence of the buds is attained due to prolonged exposure to chilling temperatures. For this, temperatures slightly above 0 °C are most effective. After the rest period, there is a period of quiescence during which external conditions may prevent growth. Exposure to warm temperatures is then required for dormancy release, which is indicated by initiation of growth (Sarvas 1974, Fuchigami et al. 1982).

Development of frost hardiness can be divided into three acclimation stages. The first stage of acclimation is induced by short photoperiod (Aronsson 1975, Christersson 1978) and is characterized by growth cessation and only a few degrees increase in frost hardiness. The second stage of acclimation is induced by low temperatures (Tumanov 1967, Weiser 1970, Proebsting 1978). In this stage, trees usually become sufficiently frost hardy to tolerate the lowest temperatures of the year. There is also a third stage of acclimation, which is induced by prolonged exposure to temperatures between –30 and –50 °C. During this stage, trees can resist temperatures as low as –196 °C. This kind of hardiness is not commonly attained in nature and is quickly lost when the temperature increases (Tumanov and Krasavtsev 1959).

The stage of bud dormancy appears to affect the acclimation processes of trees. The start of the first stage of acclimation coincides with growth cessation, and the second stage of acclimation with the rest period. Furthermore, it has been noted that during the rest period a hardy plant does not deharden even at warm temperatures. Only during quiescence can frost hardiness decrease substantially at warm temperatures (Proebsting 1963, 1970, Mac Irving and Lanphear 1967, Litzow and Pellet 1980, Fuchigami et al. 1982).

The purpose of this study was to examine the relationship between changes in bud dormancy stage and frost hardiness of the shoots of Scots pine (*Pinus sylvestris* L.) seedlings.

We thank Joann von Weissenberg for revising the English of this article.

# 2. Materials and methods

## 21. Plant material

The seedlings used in this study were grown at the Suonenjoki Commercial Nursery (62° 40' N, 27° 00' E, 130 m asl) in central Finland. The seeds, which were of local origin, were sown on June 11, 1985 into 152 cm$^3$ paper pots containing a peat-sand mixture in a greenhouse. In August 1985, all 1815 seedlings used for the experiment were transferred outdoors, where they remained until the autumn of 1986. The seedlings were grown according to normal nursery routines for the first two growing seasons; the routines included irrigation, weed control and fertilization. Fertilization was stopped at the end of August 1986.

When the seedlings were transferred to a greenhouse September 18, 1986, the temperature sum had reached 1190 d.d. (temperatures above +5 °C). The mean September temperature had been 7.7 °C, with a range of 4.3 to 14.4 °C (Fig. 1). The temperature in the greenhouse averaged 10 °C, (range 1.0–28.5 °C). The seedlings were kept under natural light conditions; in the end of September the night length was about 10 hours. While in the greenhouse, the seedlings were transplanted to larger (8 x 8 x 11 cm) containers. Throughout the experiment, irrigation was continued as necessary.

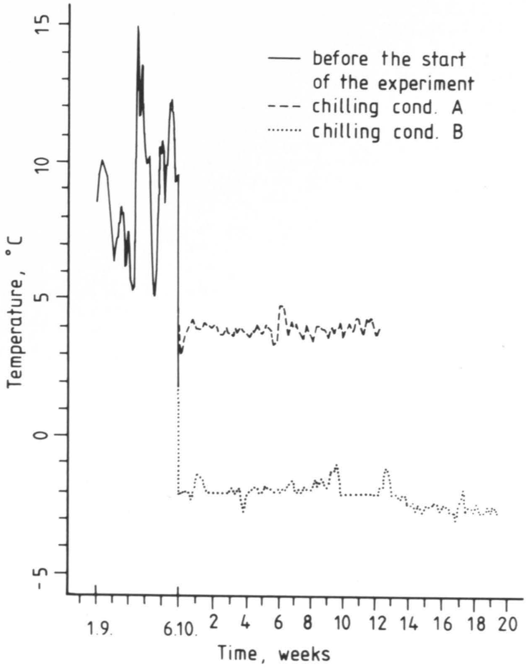

Figure 1. Daily mean temperatures before and during the experiment.

## 22. Experimental design

Seedlings were randomly divided into seven treatment groups. Beginning on October 6, 1986, the seedlings were exposed to one of two chilling temperatures, +4 °C (±2.0) or –2 °C (±1.0), in chilling chambers without illumination. Seedlings of treatment groups A and B were chilled at +4 °C and –2 °C, respectively, for the entire 19-week experiment (Figs. 1 and 2). Treatment groups C through G were transferred from +4 °C to –2 °C at biweekly intervals. Random subsamples (test groups) from the treatment groups were used to test dormancy release (n = 15) or frost hardiness (n = 60) at intervals of one to four weeks. Dormancy release was examined only in treatment groups A, B, and C.

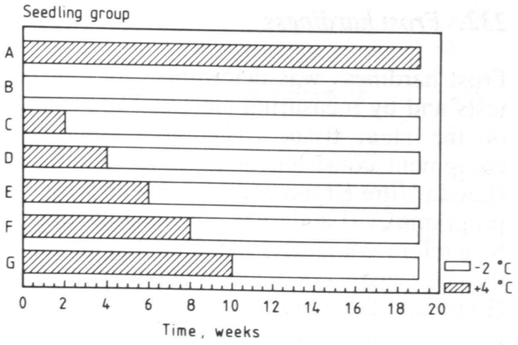

Figure 2. Chilling treatments of the seedling groups.

## 23. Measurements and calculations

### 231. *Dormancy release*

A forcing technique was used to examine dormancy release in each test group of treatment groups A, B, and C. The forcing temperature in the growth chamber was +15 °C (±3.0), with a 12-hour photoperiod and 50–100 μEm$^{-2}$s$^{-1}$ photon flux density.

Bud burst was determined by measuring terminal bud growth (±1 mm) twice a week. For days without a measured value, growth was determined by linear interpolation. A seedling was considered to have burst bud, if the total growth was more than 5 mm. The time of bud burst was determined for each seedling as the day when the growth attained ten percent of the final extension of the seedling.

Two measures of dormancy were determined for each test group: 1) dormancy release ratio (DRR), the percentage of seedlings for which dormancy was released (i.e. bud burst took place) and 2) mean days to bud burst (DBB) under forcing conditions (Hänninen 1990). These variables were analysed as a function of duration of the chilling period.

### 232. *Frost hardiness*

Frost hardiness was determined by freezing tests and by measuring electrical impedance of the stem tissue. The frost simulation equipment consisted of a refrigeration unit (Lauda Ultra Kryomate), fitted with a digital programmer (Lauda PM 350) and controlled by a micro-computer, which cooled the air of the six chambers with circulating alcohol (Repo and Pelkonen 1986). In each freezing test, ten seedlings were used at each of six test temperatures. At the coldest test temperatures the roots of the seedlings froze because the root containers were uncovered. The cooling rate in the chambers was 5.5 °C hour$^{-1}$. Test temperatures were maintained for 3 to 6 hours, with higher temperatures maintained for longer periods due to the limitations of equipment. All chambers were warmed simultaneously to room temperature at a rate of 9 °C hour$^{-1}$.

Electrical impedance of each seedling stem was measured with steel needle electrodes at a frequency of 1 kHz at room temperature before and after the freezing test. The distance between the electrodes was 15 mm.

The specific impedance was calculated by normalizing the measured impedance to a given cross-sectional area of the stem and distance between electrodes (15 mm in the present study). Frost hardiness was assessed as corresponding to a 10 Ωm lowering of the specific impedance. For a given test group, the frost hardiness was determined according to the following steps (Repo and Pelkonen 1986):

(i) The specific impedance difference Δz was calculated for each seedling of the test group as follows:

$$\Delta z = \frac{(z_2 - z_1) * A}{L} \qquad (1)$$

where

Δz = specific impedance difference Ωm
$z_1$ = absolute impedance before the freezing test Ω
$z_2$ = absolute impedance after the freezing test Ω
A = cross-sectional area of the stem $m^2$
L = distance between electrodes m

(ii) the mean value of Δz for the ten seedlings was calculated for each of the six test temperatures,

(iii) the six mean values of Δz were plotted against the test temperature and a curve was drawn through the mean values,

(iv) frost hardiness of the test group was determined graphically from the curve as the temperature corresponding to a specific impedance difference of –10 Ωm.

# 3. Results and discussion

## 31. Development of dormancy stages

At the start of the experiment, bud burst occurred in 46 % of the seedlings without any chilling beyond that received prior to the experiment. At both chilling temperatures, exposure to controlled chilling for two weeks was sufficient to break the rest of all the remaining seedlings. The mean number of days to bud burst, DBB, decreased rapidly with increasing duration of chilling (Fig. 3). DBB stabilized after eight to ten weeks of chilling. Similar results of DBB have been reported in several previous studies (Landsberg 1974, Ritchie 1984, Cannell 1985).

These results indicate that Scots pine seedlings have a rest period, during which growth is not initiated under forcing conditions. After this period the seedlings enter a stage during which growth initiation is possible in favourable environmental conditions, although with further chilling DBB still decreases gradually.

## 32. Development of frost hardiness

At the start of the experiment the frost hardiness of the seedlings was about –25 °C (Fig 4.). After two weeks, seedlings chilled at +4 °C throughout the experiment (group A) started to deharden at a rate of 0.5 °C day$^{-1}$. After eight weeks, the frost hardiness of group A stabilized at –5 °C. In contrast, the frost hardiness of group B seedlings, which were chilled at –2 °C throughout the experiment, remained at the original level until the end of the experiment. When seedlings were transferred from +4 °C to –2 °C during the dehardening period, the frost hardiness stabilized at the level it had reached at the time of transfer (groups C, D and E). When the frost hardiness of group A had reached the minimum (–5 °C), rehardening (0.3 °C day$^{-1}$) occurred after transfer to –2 °C (groups F and G).

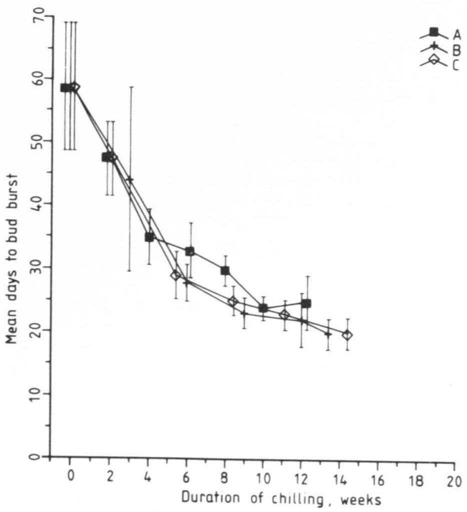

Figure 3. Mean days to bud burst (DBB) as a function of duration of controlled chilling for groups A, B, and C, described in Fig. 2. Vertical bars indicate standard deviation.

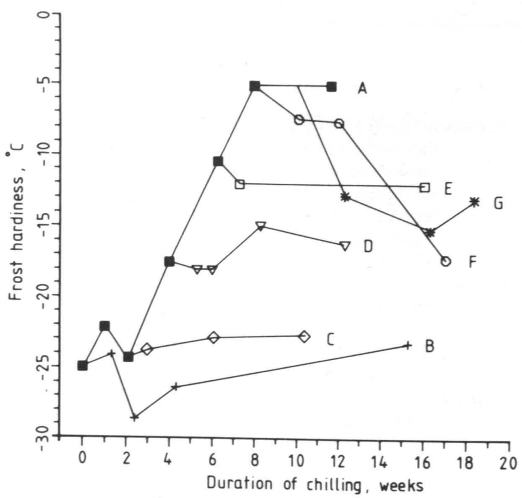

Figure 4. Frost hardiness of the seedling groups as a function of duration of controlled chilling.

## 33. Relationship between dormancy and frost hardiness

There were two times when dormancy stage and frost resistance changed simultaneously. When DRR reached 100 %, after two weeks of controlled chilling, group A began to deharden. Thus, the temperature response of frost hardiness changed when the seedlings entered the stage of quiescence. This has also been reported previously (Proebsting 1963, 1970, Mac Irving and Lanphear 1967, Litzow and Pellet 1980, Fuchigami et al. 1982). Dehardening of group A to –5 °C was complete after eight weeks at +4 °C, and the seedlings were able to reharden at –2 °C. At that time, the DBB-values were already close to their minima (Figs. 3 and 4).

These results indicate that the relationship between temperature and frost hardiness differs in different stages of the dormant period. At the onset of quiescence, the temperature range which causes dehardening is changed. Dehardening and decreasing DBB during quiescence indicate that the seedlings are entering the next growing period.

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