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Rehardening potential of Scots pine seedlings during dehardening

Tapani Repo

TIIVISTELMÄ: MÄNNYN TAIMIEN KYKY KARAISTUA UUDELLEEN SUVEENTUMISEN AIKANA

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The ability of one- and two-year-old Scots pine (*Pinus sylvestris* L.) seedlings to reharden during the dehardening period was studied. Naturally hardened quiescent seedlings were preconditioned at 0 °C for ten days and then placed in chambers at different forcing temperatures with different light regimes. The forcing periods were followed by cool periods. Changes in frost hardiness were monitored at intervals using freeze tests of whole plants. Frost hardiness was assessed by three methods: impedance, survival and growth retardation. Dehardening seemed to be a partially reversible process, i.e. in some growing conditions slight rehardening was found.

Tutkimuksessa selvitettiin yksi- ja kaksivuotiaiden männyn (*Pinus sylvestris* L.) taimien kykyä karaistua uudelleen suveentumisen alettua. Luonnon oloissa karaistuneita kvaiesenssivaiheessa olleita taimia pidettiin aluksi 10 vrk 0 °C, jonka jälkeen ne siirrettiin eri hyötöoloihin 10–14 vuorokaudeksi. Hyötöjakson jälkeen taimet siirrettiin takaisin viileisiin kasvuoloihin. Taimien pakkaskestävyyttä seurattiin koko kasvin pakkastestein. Pakkaskestävyyden määrityksessä käytettiin kolmea menetelmää: verson impedanssimittaus, visuaalisten vaurioiden arviointi ja pakkaskäsittelyn jälkeisen kasvun mittaus. Suveentuminen osoittautui osittain reversiibeliksi tapahtumaksi, ts. eräissä kasvatusoloissa havaittiin uudelleen karaistumista.

Keywords: frost resistance, impedance, *Pinus*, quiescence, acclimatization.
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1 Introduction

Considerable daily fluctuations in temperature occur in boreal forests at the time when the trees are still in a frost-hardened, quiescent stage. Subsequent cold (below –40 °C) and warm periods (a few degrees above zero), with durations of several days or even weeks, are possible in winter. Diurnal changes in temperature are more frequent and more severe for plants in spring when the sun warms them considerably during the daytime and the night temperature can be as low as –20 °C. Trees must have both sufficient ability to tolerate low temperatures, and also the ability to tolerate or resist desiccation due to transpiration and evaporation.

Previous studies have shown that dehardening of trees is driven mainly by temperature (van den Driessche 1969, Greer and Stanley 1985). For example, when hardened Scots pine seedlings were put into a warm environment in winter, frost hardiness was lost with a time constant of several days (Repo and Pelkonen 1986). In addition, a stable value for frost hardiness was reached at a warm temperature. Some earlier reports have also shown that dehardening may be a reversible process and that in some plant species, after a period of warm weather, rehardening may occur during subsequent cold weather (e.g. Proebsting 1963, Sakai 1966, Mac Irving and Lanphear 1967, Howell and Weiser 1970, Hamilton 1973, Cannell and Sheppard 1982, Dale and Heiberg 1984, Tinus and Burr 1989). This phenomenon is largely unknown in boreal trees. The aim of this study was to determine whether, in controlled growing conditions, rehardening occurs in Scots pine (*Pinus sylvestris* L.) seedlings during the dehardening period, and if so, whether rehardening is completely or partially reversible.

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2 Material and methods

2.1 Experiments

Experiment 1: One-year-old Scots pine paperpot seedlings of local origin were grown at the Suonenjoki Commercial Nursery (62°40' N, 27°00' E, altitude 130 m asl). The seedlings were randomly selected from the seedling bed and were conditioned outdoors under the snow in the nursery before the start of the experiment. The quiescent seedlings were transferred on 8 March 1985 (late winter) to a growth chamber at 0 °C (±1 °C, humidity ca. 90 %) in darkness (phase 0, Fig. 1 A). After ten days, the temperature was increased to +8 °C (±1 °C, humidity 60–80 %) (phase 1). At 14-day intervals the temperature in the dark growth chamber was then varied from 1 to 8 ± °C (phases 2 and 3). A freezing test was performed one day after the start of phase 1 and repeated at three- to seven-day intervals.

Experiment 2: The material consisted of two-year-old Scots pine seedlings (Enso pots) grown at the Suonenjoki nursery. The seedlings, which were overwintering under the snow, were of local origin. On 24 March 1986, when the pots were still covered with snow, they were transferred indoors to 0 °C (±1 °C) (phase 0, Fig. 1 B). After ten days, the temperature was raised to 13 °C (phase 1) (humidity 70–90 %). After an additional twelve days, one lot of seedlings was returned to cool growing conditions (–1 ±1 °C, phase 2); another lot was left at 13 °C. The seedlings were illuminated with fluorescent tubes (Philips TL65-80W/55RS and Osram 65W/20R), which gave a photon flux density of about 50 µmolm^{-2}s^{-1}, during the entire experiment. Day/night length was 12/12h. The frost hardiness of the previous year's shoot was estimated at the beginning of the experiment and at frequent intervals afterwards.

The bud burst of seedlings was monitored during phase 1 (+13 °C). The date of bud burst was determined as the time instant when the average extension of the shoot was 10 % of the final extension. The effect of the cool period (phase 2) on bud burst and growth was determined by removing lots of 15 seedlings from –1 °C at intervals and putting them back at 13 °C.

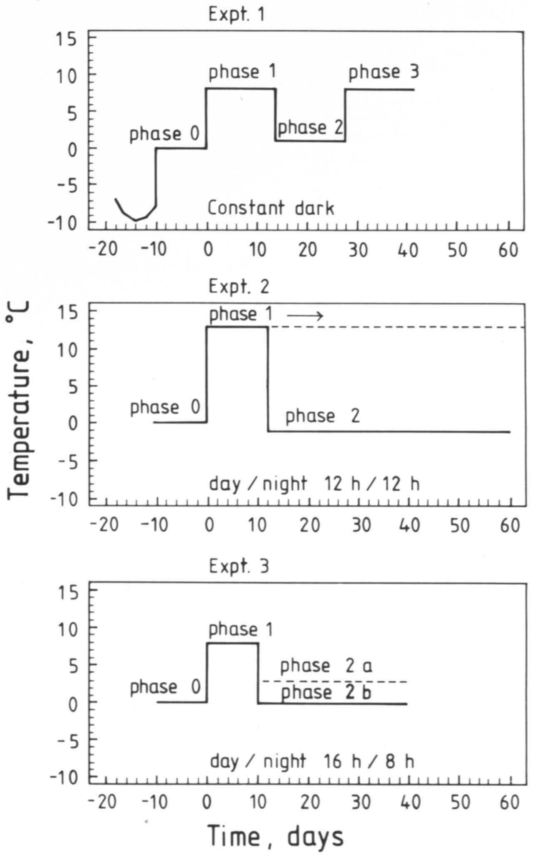

Figure 1. Environmental stimuli in Experiments 1, 2 and 3. For times less than –10 days, temperature is that of the snow covering the seedlings in the field before the start of the experiment. For times greater than –10 days, temperature is that of air in controlled conditions. Preconditioning is indicated as phase 0.

Experiment 3: One-year-old Scots pine seedlings of local origin were grown in paper pots at the Suonenjoki nursery. The seedlings were hardened in field conditions. At the beginning of January 1988, the snowcover was removed and the seedlings were transferred to 0 °C (±1 °C) in darkness for 10 days (phase 0, Fig. 1 C). Thereafter the temperature was raised to 8 °C (±1 °C) (phase 1). The seedlings were illuminated with fluorescent tubes (Philips TL65-80W/55RS), which gave a photon flux density of about 45 µmolm^{-2}s^{-1}. Day/night length was 16/8 h and relative humidity about 80 %. After 11 days in 8 °C, one lot of seedlings was removed to 3 °C (±1 °C) (phase 2a). The seedlings were illuminated with mercury-halogen lamps with a photon flux density of about 120 µmolm^{-2}s^{-1}, and the day/night length was 16/8 h. Another lot was left in the same conditions as in phase 1, except that the temperature was set at 0 °C (±1 °C) (phase 2b). Phases 2a and 2b each lasted about one month. For plants of each growing condition the frost hardiness was tested about once a week.

2.2 Assesments of frost hardiness

In all experiments, the frost hardiness of the seedlings was estimated by three techniques using a whole-plant freezing test: 1) the temperature response of the specific impedance difference (Repo and Pelkonen 1986), 2) scoring of visual damage, and 3) retardation of shoot elongation. During the tests, the rates of cooling and warming of the freezer were about 6 and 7 °C/h, respectively. The minimum temperature was maintained for about 4 h. Usually six different exposure temperatures were used during a single freezing test. Eight seedlings were exposed to each temperature during Expt. 1, and 10 seedlings during Expts. 2 and 3. No artificial ice inoculation was used during freezing. The seedling containers were set in moist sand to protect the roots against frost.

Impedance-estimated frost hardiness ($LT_{-10\Omega m}$)

All impedance measurements were taken at 20 ±2 °C, before and after the whole-plant freezing test. The impedance measuring device in Expt. 1 was a Finnish prototype, which uses sine input voltage of 2.0 V (U_{rms}). The impedance was measured at a frequency of 1 kHz. In Expt. 2 the device was the Conditiometer AS 1 (Forst+Holz, Techn. Instrumente and Geräte, Austria), which uses a square input voltage (U_{pp}=1 V) and a frequency of 33 Hz. In Expt. 3 the impedance was measured by a voltage divider with a front resistance of 39 kΩ. Sine input voltage (f=1 kHz) was 350 mV (U_{rms}). Input impedance of the voltage measuring device was 10 MΩ. The steel needle electrodes (diameter = 0.7 mm) were pushed through the stem during the measurements.

The standard error of the impedance-estimated frost hardiness was calculated in cases where measured data allowed sigmoid curve fitting. The calculus was carried out by expressing the specific impedance difference as a logistic sigmoid function of the exposure temperature and evaluating the inverse function at a given value of the specific impedance difference (–10Ωm). The error variance between the estimated and the measured frost hardiness was calculated using the estimated parameters, their standard deviations, and correlations (Repo and Lappi 1989).

Assessment of frost hardiness according to visual damage (LT_{10} and LT_{50})

After frost exposures and impedance measurements, the seedlings were transferred into a greenhouse where the temperature was about 20 °C. In addition to natural illumination, mercury-halogen lamps with a photoperiod of 16/8h (day/night) were used. After the shoot and needle elongation had stopped, damage was scored visually. In Expt. 1 the seedlings were classified in two groups in which the damage symptoms were as follows:

1. No visual damage, or abnormalities in the shape or occurrence of new needles, or stunted growth of the new shoot.
0. Seedling dead.

The mean of the scored values were calculated for each temperature classes. Accordingly, a temperature response curve of damage was obtained. The LT_{10}-value (90 % survival) at a given moment was found with linear interpolation between the means of the scored values.

In Expt. 2 damage to the seedlings were classified in two groups in which the damage symptoms were as follows:

1. No visible damage, or abnormalities in either the shape or occurrence of new needles.
0. Whole seedling dead, or no growth in apical or lateral shoot, or new leader shoot dead but growth in lateral shoots.

LT_{50}-value (50 % survival) was obtained with a procedure similar to that for LT_{10} in Expt. 1.

In Expt. 3 damage to the seedlings was scored as follows:

1. Seedling living/dead – 1/0.
2. New apical shoot living/dead – 1/0.
3. Amount of needle damage on the new shoot was scored in five intervals of 20 % each.

LT_{50}-values for groups 1 and 2 were obtained by a procedure similar as in Exp. 1 and 2. The corresponding value for the needles was obtained by linear interpolation between the mean values of intervals by exposure temperatures.

Growth-estimated frost hardiness (LTG_{10})

In all experiments the length of the new shoot was measured when damage was scored. The mean value for shoot elongation at the two warmest frost treatment temperatures (mean of 16 seedlings in Expt. 1 and a mean of 20 seedlings in Expts. 2 and 3) was taken as the reference or "undamaged" growth. Where growth had decreased 10 % and 50 %, the temperature equivalents were assessed from the curves. In the following text these are called growth-estimated frost hardiness and are indicated as LTG_{10} and LTG_{50}, respectively.

3 Results

Experiment 1: At the time of the first freezing test (10 days at 0 °C and 1 day at 8 °C) the impedance-, growth- and survival-estimated frost hardiness were –24 °C ($LT_{-10\Omega m}$), –26 °C (LTG_{10}) (Fig. 2) and –33 °C (LT_{10}), respectively. In the subsequent freezing tests, minimum temperatures were usually too high to permit assessment of LT_{10}. In addition, in all tests damage was too slight to cause 50 % decrease in shoot elongation (LTG_{50}). Dehardening started at 8 °C and progressed according to a linear curve fitted at a rate of 0.7 °C/day ($LT_{-10\Omega m}$) and 1.0 °C/day (LTG_{10}). At the end of the first phase, the frost hardiness was about –15 °C.

Frost hardiness as determined by the impedance method increased; i.e. rehardening occurred during the following cool period (14 days at 1 °C). Frost hardiness reached a stationary level of about –18 °C. In contrast to this result, no clear evidence of rehardening was found in the LTG_{10}-curve.

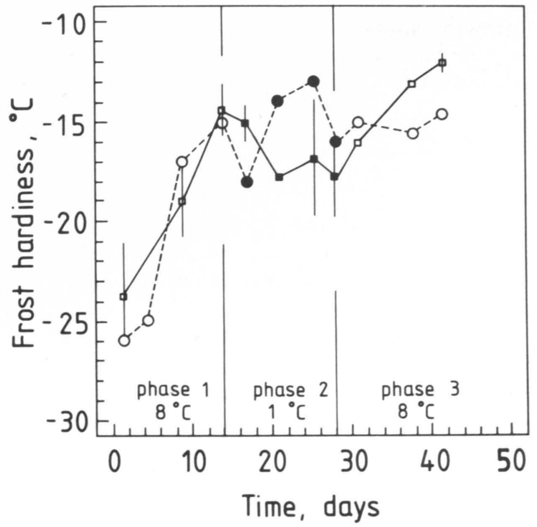

Figure 2. Frost hardiness of one-year-old Scots pine seedlings in Expt. 1; impedance- ($LT_{-10\Omega m}$) (–□–) and growth-estimated (LTG_{10}) (–○–) frost hardiness. The cool period (phase 2) is indicated by closed symbols. The bars indicate standard error in cases where measured data allowed a sigmoid curve to be fitted to the data. Temperature phases are indicated across the bottom of the graph.

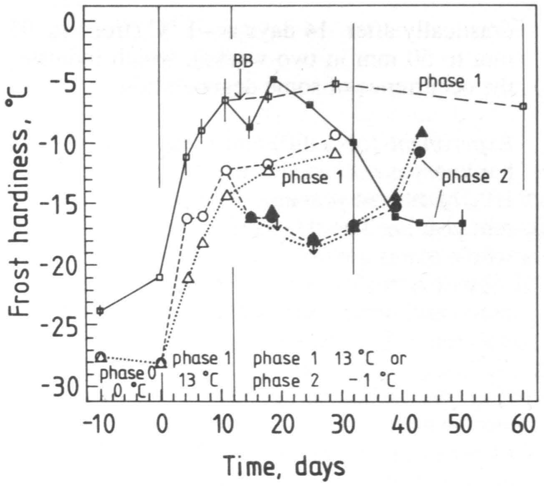

Figure 3. Frost hardiness of two-year-old Scots pine seedlings in Expt. 2; impedance- ($LT_{-10\Omega m}$) (–□–), survival- (LT_{50}) (–△–) and growth-estimated (LTG_{50}) (–○–) frost hardiness. The cool period (phase 2) is indicated by closed symbols. The bars indicate standard error in cases where measured data allowed a sigmoid curve to be fitted to the data. Temperature phases are indicated across the bottom of the graph. Bud burst in 13 °C is indicated as BB.

When the temperature in the growing chamber was raised to +8 °C (phase 3), dehardening as estimated with the impedance method started again with a rate of 0.4 °C/day; whereas the LTG_{10}-value remained at about –15 °C for the last two weeks. The experiment was stopped during the third phase when the frost hardiness ($LT_{-10\Omega m}$) was –12 °C. The seedlings were then moved to the greenhouse (≈ 20 °C) where bud burst took place four days later.

The mean shoot elongation of the undamaged seedlings used as references for estimation of LTG_{10}-value remained about the same (ca. 70 mm) or even increased slightly during the study period, which suggests that degeneration had not occurred.

Experiment 2: At the beginning of the experiment the impedance-, survival- and growth-estimated frost hardiness were –24 °C ($LT_{-10\Omega m}$), –28 °C (LT_{50}) and –28 °C (LTG_{50}), respectively (Fig. 3). These levels were retained during the preconditioning period at 0 °C. When the seedlings were taken to +13 °C (phase 1), dehardening proceeded at a maximum rate of ca. 2 °C/day, as assessed by each of the three methods. At the end of this phase (12 days at 13 °C), impedance-, survival- and growth-estimated frost hardiness were –6.4 °C, –14.2 °C and –12.0 °C, respectively. The seedlings that remained in 13 °C (phase 1) continued to deharden, and attained a minimum hardiness of –5°C ($LT_{-10\Omega m}$), –11 °C (LT_{50}), and –9 °C (LTG_{50}), respectively (Fig. 3).

During the following cold period (–1 °C) (phase 2), the impedance-estimated frost hardiness first seemed to have a latent period of about 12 days before the start of rehardening (Fig. 3). When frost hardiness was based on visual damage or growth retardation, slight rehardening (ca. 3 °C) was found in 4 days at –1 °C. After a prolonged period of chilling, when the impedance method indicated increased frost hardiness, the other two methods showed the opposite. When the reference seedlings were taken from chilling conditions to the greenhouse, however, the mean shoot elongation of these seedlings decreased drastically after 14 days at –1 °C (from ca. 95 mm to 50 mm in two weeks), which indicates the occurrence of some degeneration.

Experiment 3: No difference was found in frost hardiness as estimated with LT_{50} of whole plants, LT_{50} of current year apical shoot or LT_{50} of current year needles. Thus only the LT_{50} values for whole plants are presented.

Frost hardiness of the seedlings during the preconditioning period (10 days in 0 °C) was much like that in Expts. 1 and 2; –24 °C ($LT_{-10\Omega m}$), –27 °C (LT_{50}) and –27 °C (LTG_{50}) (Fig. 4). Dehardening at +8 °C started more slowly than in previous experiments. The slope of the linear regression fitted to the impedance data was 0.4 °C/day, whereas survival- and growth-estimated frost hardiness had a latent period of about 6 days. Thereafter the plants dehardened rapidly (ca. 1.4 °C/day) to –17 °C.

According to the impedance data, no rehardening was found during the following cool period at either 0 °C or 3 °C (Fig. 4, phase 2b and Fig. 5, phase 2a). In fact, frost hardiness seemed to have reached a stationary value of –18 °C at 0 °C (Fig. 4), whereas dehardening slightly continued at +3 °C (Fig. 5). The latter result was supported by survival- and growth-estimated data. On the other hand, at 0 °C slight rehardening was found when survival and growth indices were estimated. The mean shoot elongation of the 20 seedlings used as the reference for growth-estimated frost hardiness decreased slightly toward the end of the experiment.

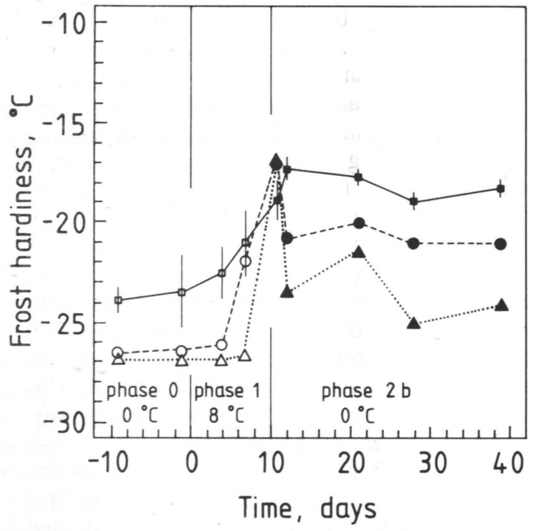

Figure 4. Frost hardiness of one-year-old Scots pine seedlings in Expt. 3 ending with phase 3 at 0 °C (Fig. 1). Impedance- ($LT_{-10\Omega m}$) (-□-), survival- (LT_{50}) (-△-) and growth-estimated (LTG_{50}) (-○-) frost hardiness. The cool period (phase 3) is indicated by closed symbols. The bars indicate standard error in cases where measured data allowed a sigmoid curve to be fitted to the data. Temperature phases are indicated across the bottom of the graph.

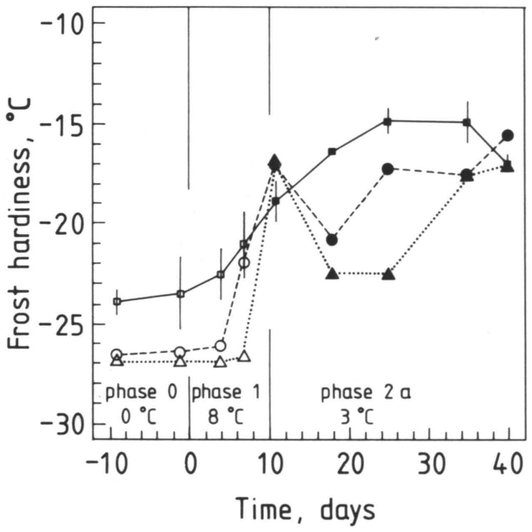

Figure 5. Frost hardiness of one-year-old Scots pine seedlings in Expt. 3 ending with phase 2 at 3 °C (Fig. 1). Impedance- ($LT_{-10\Omega m}$) (-□-), survival- (LT_{50}) (-△-) and growth-estimated (LTG_{50}) (-○-) frost hardiness. The cool period (phase 2) is indicated by closed symbols. The bars indicate standard error in cases where measured data allowed a sigmoid curve to be fitted to the data. Temperature phases are indicated across the bottom of the graph.

4 Discussion

Due to technical problems, the device for measuring impedance had to be changed between experiments. Known resistors were used to calibrate the devices to about the same value. In addition, calculation of the differential value of post- and prefreezing impedances reduced the error due to the different methods.

Scoring of visual damage differed slightly from experiment to experiment. In Expt. 1 assessment of frost hardiness was based on scoring the survival of the whole plant. Exposure temperatures were usually too high to obtain LT_{50}- or even LT_{10}-value. LT_{50}-value as estimated in Expt. 2 refers to frost hardiness of the apical bud or new leader shoot if bud burst had occurred. On the other hand, the LT_{50}-value in Expt. 2 corresponds to the LT_{50}-value of the new apical shoot in Expt. 3. The scoring procedure in Expt. 1 might have overestimated hardiness compared to the results in Expts. 2 and 3.

Survival-estimated and growth-estimated frost hardiness followed each other quite closely during dehardening in Expts. 2 and 3 (Figs. 3 and 4, phase 1) and also during rehardening in Expt. 2 (Fig. 3, phase 2) but they differed during the "rehardening period" in Expt. 3 (Fig. 4, phase 2b and Fig. 5, phase 2a). During the dehardening phase, these values were 0–10 °C lower than impedance-estimated frost hardiness, and the difference changed with hardiness level.

The estimate of frost hardiness by a 10 % decrease in shoot elongation cannot be regarded as very accurate because a slight variability in "natural" growth may have influenced the estimated value. On the other hand, the estimate by a 50 % decrease in growth agreed well with the changes in hardiness assessed with other methods.

The survival- and growth-estimated frost hardiness at 11 days (–17 °C) (Figs. 4 and 5) was lower than would be expected. Nor do impedance data support these data. The temperature response curves from which these points were obtained ascend smoothly. On both curves one point is located in the "critical range", but these points have quite high standard deviations (survival = 0.4±0.5, growth = 27±39 mm). A slight increase in the above mean values would change the response curves so that the frost hardiness would be higher than obtained currently. It may be that some factors other than frost damage affected the seedlings in this group. Otherwise rehardening would have progressed rapidly at the beginning of the cool period.

The developmental stage of the seedlings at the beginning of the "rehardening phase" in Expt. 2 differed from the corresponding stage in Expts. 1 and 3. At the end of phase 1 in Expt. 2 (Fig. 3) the apical buds were found to be slightly elongated, whereas in the other two experiments no elongation was found at the end of the first forcing period. Thus, owing to the different developmental stages, the rehardening response in Expt. 2 could be expected to differ from that in Expts. 1 and 3.

Bud burst has been regarded as a critical point event for rehardening capability (Fuchigami et al. 1982, Cannell and Sheppard 1982). Thus, it was surprising that a slight increase in hardiness was found in Expt. 2 (Fig. 3, phase 2), as estimated by survival and growth, and, after a latent period, also with impedance-estimated frost hardiness. This can be considered to be partly an artifact, however, because growth potential decreased during the cold period (at least after 22 days). No decrease was found in specific impedance values before freezing, which would have indicated damage in stem tissues. The results may be interpreted as indicating that buds and needles suffered damage during the prolonged cold period (–1±1 °C), which appeared as stunted growth, but the stems were able to reharden.

The seedlings in Expts. 1 and 3 were not exactly at the same stage of hardiness when the "rehardening phase" (Fig. 2, phase 2 and Fig. 4, phase 2b) began. This might explain the differences in rehardening response. In Expt. 1 hardiness was –15 °C at the beginning of the "rehardening phase", whereas the respective value in Expt. 3 was –17.5 °C. In Expt. 1 during the following cool period rehardening leveled off at –18 °C (Fig. 2, phase 2), which was about the value reached at the end of the "dehardening phase" (phase 1) in Expt. 3. Accordingly, substantial rehardening could not be expected at 0 °C in Expt. 3 (phase 2b), and the increase in hardiness (to –18.5 °C) was found to be only 1 °C. We can conclude that –18 °C is about the stationary value to which one-year-old Scots pine seedlings can reharden to at 0 °C, after dehardening has begun.

The large difference between growing temperature and respective hardiness indicates that Scots pine seedlings can tolerate large temperature changes without damage. For example, at the end of phase 0 in Expts. 1, 2 and 3 (Figs. 2, 3 and 4) the difference in temperature was more than 20 °C. The difference seems, however, to depend on the stage of development and decreases as dehardening progresses. For example, it was less than 20 °C at the end of phase 2 in Expt. 1 (Fig. 2) or phase 2b in Expt. 3 (Fig. 4). Due to the large difference between growing temperature and the corresponding frost hardiness, we might conclude that the plants do not have much use for their rehardening capability. In the field, however, it is not unusual to have short-term (days to weeks) temperature variations that are greater than 20 °C, especially in the spring after dehardening has begun. Thus it is obvious that rehardening capability is important for avoidance of frost damage.

The temperature in the controlled growing conditions constituted only a narrow range of the actual temperatures that occur in spring. In field conditions, temperature fluctuates within days and between days, which is in contrast to the constant temperatures for several days and weeks used in this study. Light conditions in the field conditions also differ from those in our study. High photon flux densities are usually associated with day/night temperature fluctuations. Different plant organs (buds, needles, stems) may respond differently in fluctuating conditions. These facts should be taken into account when the results of this study are applied to field conditions.

The experiments were started when the chilling requirement for rest break was satisfied and the seedlings were quiescent. It is obvious that the reversibility of frost hardiness changes during dormancy (cf. Tinus and Burr 1989). Sakai and Larcher (1987, p. 109) state that in late winter, during post-dormancy, plants are more easily dehardened and less easily rehardened. Our results support this conclusion.

The reversibility of dehardening in Scots pine seedlings became quite clear in this study, but dehardening proved to be only partially reversible. The level of frost hardiness attained during preconditioning at 0 °C was about the same in the different experiments when the results of the same assessment methods were compared. After dehardening had proceeded at a higher temperature and the seedlings were returned to 0 °C, the level of frost hardiness was not the same as at the end of the preconditioning period in any of the experiments with any of the methods used.

The results of this study gave new information for modeling of frost hardiness. In a previous study (Repo et al. 1990) frost hardiness was assumed as a first-order process driven by air temperature. According to this model type frost hardiness would reach a stable value when plants are taken to a constant temperature, according to a first order differential equation with a time constant. In this type of model, dehardening is assumed to be a reversible process, i.e. an increase in frost hardiness (rehardening) occurs independently of the stage of dehardening or the stage of development.

The results of this study partly support the assumption of the first-order type of model as is the case during dehardening. Stationary levels of frost hardiness are reached during dehardening and rehardening, although partially reversibly in the latter case. The slow response in the forcing conditions of Expt. 3 (Fig. 4) indicates that dehardening could be of second order. The dependency between the stable value reached and the growing temperature, and perhaps the rate of response, also seems to vary depending on the degree of hardiness, i.e. the stage of development (cf. Dale and Heiberg 1984).

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