# Frost hardening and dehardening in *Abies procera* and other conifers under differing temperature regimes and warm-spell treatments

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*Summary*

Frequent bud frost damage in cultivation of *Abies procera* Rehder and pending climate changes are the background for this study of cold hardiness under varying acclimation regime (in closed-top chambers) and experimental warm spells during the cold season. $LT_{50}$ values were established by freezing tests at different times of year. Damage and deaths were assessed on leader buds, subapical lateral buds, needles and cambium. Minor parallel experiments involved *Abies nordmanniana*, *Picea abies* and *Picea sitchensis*. Lower acclimation temperatures resulted in deeper frost hardiness during late autumn but less during spring, compared with ambient temperature controls. Elevated temperatures resulted in less deep frost resistance. Apical buds generally developed deeper frost hardiness than lateral buds but less deep than the cambium, varying with species, however. Frost damage in buds ranged from death over partially destroyed bud contents resulting in distorted shoots to buds seemingly remaining dormant. Responses to warm spells differed with duration, timing and species, ranging from dramatic decrease in frost hardiness with or without subsequent recovery to no reaction. Furthermore, the reactions did not show any clear relation to dormancy level. For *A. procera*, exposure to fluctuating temperatures appeared to be particularly problematic. This explains why this species develops best in coastal climates, and in sites sheltered from temperature extremes either by hedging, a winter snow cover, or topography. The Christmas tree production will suffer severely on sites with harsh temperatures due to losses of lateral and terminal buds, which destroy the crown symmetry. Clipping of greenery is less influenced by frost damages, although the development of normal branch whorls is often disturbed.

## Introduction

Phenology and dormancy patterns of trees are adapted to the conditions of their natural habitat (Chuine and Cour, 1999). Hence, species and provenances that are cultivated far from their origin may display problems in seasonal timing, which can lead to irregular bud breaking, poorly formed shoots or loss of buds. Successful cultivation requires a deeper analysis of the critical environmental stimuli that are needed to carry trees through the annual cycle, such as dormancy and cold hardiness. The topical interest in climate change further emphasizes the relevance of such knowledge.

Noble fir (*Abies procera* Rehder) is native to the Cascade Mountains, north-western USA, and

is extensively used for Christmas tree and greenery production not only in America (Franklin, 1990) but also in Ireland, United Kingdom and Denmark. Outside its natural range Noble fir does best in Ireland, Scotland and England, whereas in Denmark the leader bud as well as lateral buds are often damaged during winter and early spring. Such disturbances destroy crown symmetry and thus reduce the ornamental value. Problems with lateral buds decrease with increasing tree age and tend to disappear when the trees are 2–4 m tall, but problems with the leader bud tend to persist even in older trees.

Being a mountain species from lower latitudes (44–48° N), is it reasonable to assume that the annual cycle of *A. procera* is more dependent on temperature than on day length controls. In its natural habitat, Noble fir has a shorter growing season than in Denmark, and young trees are normally covered by snow during winter. It ranks among the deep dormant species (Murray *et al.*, 1989). McKay *et al.* (1997) and Nielsen found that Noble fir benefits from chilling as late as in March for dormancy release (C.C.N. Nielsen, unpublished data).

Cultures on northern slopes, in the shadow of neighbouring old stands, and on hilltops usually suffer little bud damage, compared with trees on southern slopes and in valleys and plains (S. Sørensen and C.C.N. Nielsen, personal observation). This suggests that exposure and temperature fluctuations are important factors in bud frost damage.

During November and January, Nielsen *et al.* (1996) found comparable levels of frost hardiness in *A. procera* and other conifers (*Abies nordmanniana*, *Picea sitchensis* and *Picea abies*) in the upper crown of old trees. However, at the beginning of March, *A. procera*, together with Sitka spruce, was significantly more frost sensitive than the others and showed high mortality in Danish cultures after heavy frost (−18°C) in late April 1991. Although frost damage to buds in *A. procera* is usually assumed to occur during winter, these observations point towards autumn and early spring as the critical times.

As a climate change towards higher winter temperatures is under way, winter frost damage in *A. procera* might be expected to become less of a problem in years to come. However, if the climate change is accompanied by more unstable weather, as some prognoses suggest, the conditions for obtaining and maintaining cold hardiness during the winter season might be disturbed. The purpose of this work is to improve our understanding of cold hardiness as a function of the temperature regime, specifically focusing on temperature fluctuations, and the interaction between season and unusually warm weather periods.

## Material and methods

### Plant material and species

Experiments were carried out during the winter seasons 2000/2001, 2001/2002, 2002/2003, in the following denoted year 1, 2 and 3. In all years, bare-rooted 2-year-old seedlings of *A. procera* were potted during spring in 250 $cm^3$ containers with a peat-based substrate. The pots were grown at ambient conditions until September when they were randomly allocated to the acclimation regimes (Table 1). Testing for frost hardiness began in October. A slow release fertilizer was added 2 months after potting. Optimum irrigation of the plants was attempted at each site.

Two-year seedlings of *A. nordmanniana* Spach, *P. abies* (L.)H.Karst. and *P. sitchensis* (Bong.)Carrière were included for comparison in some of the regimes and tests, and in year 3 *A. nordmanniana* doubled in all treatments (Table 1). Seed sources were *A. nordmanniana*, 'Ambrolauri', *A. procera*, 'Linå Vesterskov' (year 1) and 'Overgård' (year 2 and 3), *P. abies*, 'Lundbæk' (year 1) and 'Buderupholm' (year 2), *P. sitchensis*, 'Silkeborg Lystrup'.

### Acclimation regimes

At the nursery of the University Arboretum in Hørsholm (52° 15′ N, 12° 29′ E), two outdoor sites represented two extremes in terms of exposure (Table 1). The ambient 'A0' site was lightly wind sheltered but exposed to whole-day sun. Within the forest, a 400-$m^2$ gap was used for a windsheltered 'Af' site, protected from direct sunlight. Next to the 'A0' site, temperature-controlled closed-top chambers (CTC) were set up to provide different temperature regimes: (1) ambient temperature, to establish a comparison with A0 ('A0$_{CTC}$'), (2) 5° above ambient temperature ('A + 5') and (3) 5° below ('A − 5'). A soil temperature sensor and a heat cable

*Table 1*: Acclimation regimes

| Acclimation regime | Temperature | Site | Insulation of root container | Year | Species |
|---|---|---|---|---|---|
| A0 | Ambient | Outdoor, exposed | + | 1, 2 | *Abies procera, Abies nordmanniana, Picea abies, Picea sitchensis* |
| A0$_{ctc}$ | Set to ambient | Controlled temperature chamber | + | 2 | *A. procera* |
| Af | Ambient, forest | Outdoor, no direct sunlight | + | 2 | *A. procera* |
| A − 5 | Set to ambient minus 5°C | Controlled temperature chamber | + | 1, 2 | *A. procera* |
| A + 5 | Set to ambient plus 5°C | Controlled temperature chamber | + | 1, 2 | *A. procera* |
| FF | Set to average yearly trend | Controlled temperature chamber | − | 3 | *A. procera, A. nordmanniana* |
| W | Constant +12°C during winter | Green house | − | 1, 2 | *A. procera* |
| W+ | Constant +20°C during winter | Green house | − | 1, 2 | *A. procera* |

were placed below the pots in A − 5 to keep the ground above −1°, in order to prevent accidental root frost. In year 3, the fluctuation free (FF) regime was set according to a 30-year average temperature trend, temperature set points changed weekly, in order to have well acclimated material for autumn warm-spell treatments. Two greenhouse cells close to the CTC's were arranged with constant minimum temperatures of +12 and +20°C ('W' and 'W+'). Root containers were insulated with wood chips in all sites except site FF, W and W+. Air temperature loggers were available on all sites and the soil temperature was monitored in A0 and Af at a depth of 100 mm.

*Warm-spell treatments*

Trees that were given warm-spell treatments were for the defined period moved into either W or W+, the intended temperature raise being 10–12°C. After warm-spell exposure, they were either returned to the acclimation site or freezing tested immediately (Table 2).

*Freezing tests and LT$_{50}$ calculation*

Plant material was tested in a 1 m³ Weiss frost chamber. Testing was carried out at four (year 1

only three) different temperatures, each receiving nine replicate trees. (In year 3, mortality during the growth season prevented consistent use of nine trees, however.) The test ran over 1 day for each test temperature. The program ensured a gradual change during the first 2 h from ambient temperature to +2°C. The material was left to adapt for another 2 h, and the temperature subsequently reduced over the next 4 h until the test level was reached. The plant material was kept at the test level for 2 h, and the temperature then again raised gradually to 2°C during a 4-h period. Finally a slow thawing was allowed at 2°C during 4 h.

Whole seedlings were used as test units, in contrast to Nielsen *et al.* (1989, 1996), who used cut-off twigs. Frost hardiness of aboveground parts was tested while the root and soil were insulated. The containers were kept in double-walled boxes and the space between walls being filled with fine wood chips. The surface of these boxes was covered with sheats of Styrophoam™ clasping the stem. In this way, frost damage to the roots was prevented.

Literature suggests several ways to estimate LT$_{50}$ values. Most methods assume a sigmoid s-shaped accumulative mortality function. Because mortality rates did not always develop gradually with increasing test temperature, and in particularly due to the relative few test temperatures, we

*Table 2*: Warm-spell treatments

| Abbreviation | Description | Test year |
|---|---|---|
| Regular | No warm-spell treatment | 1, 2, 3 |
| WSFeb | February 12–21. From A0 to W/W+ | 1 |
| WSMar1 | March 2–12. From A0 to W/W+ | 1 |
| WSMar2 | March 20–30. From A0 to W/W+ | 1 |
| WSFeb 3/6/9 | 3, 6 or 9 days, February (all ending on 11/2). From A0 to W/W+ | 2 |
| WSMar 3/6/9 | 3, 6 or 9 days, March (all ending on 11/3). From A0 to W/W+ | 2 |
| eWSOct | Moved from A0 to A − 5 during October 3–17. At site W+ October 17 to November 5. Returned to A0 at November 5 | 2 |
| eWSNov | Moved from A0 to A − 5 during November 5–26. At site W+ November 26 to December 12. Returned to A0 at December 12 | 2 |
| WSOct | October 15 to November 1. From FF to W/W+ | 3 |
| WSNov | October 29 to November 12. From FF to W/W+ | 3 |

chose to use the probit analysis (Finney, 1971) in the form presented by Larsen (1976). This analysis is developed for dose–response test with few test levels. This sometimes provided mortality sequences (e.g. 100, 90, 60 and 30 per cent) without clear asymptote values. Non-linear fitting of this kind of data is most difficult, often leading to qualitative assumptions about model shape or asymptotes or leading to weighed fitting. Instead of focusing model fitting and $LT_{50}$ estimation on the values closest to 50 per cent mortality (as done by interpolation or by weighed non-linear fitting), the probit model weighs the lowest and highest test temperature levels equal to the intermediate temperature levels. In order to avoid mortality sequences without objective upper and lower asymptotes, the four test temperatures were chosen with fairly large intervals (e.g. −20, −27, −34, −40°C during mid-winter and −3, −8, −13, −22°C during spring). This, however, made the $LT_{50}$ estimates less accurate, a reason for us to focus a great deal on the statistical tests as described below.

$$LT_{50} = \exp\langle[(M_1 - 0)\times\ln(T_1 - T_{min})] + $$
$$[(M_2 - M_1)\times\ln(T_2 - T_1)] + $$
$$[(M_3 - M_2)\times\ln(T_3 - T_2)] + $$
$$[(M_4 - M_3)\times\ln(T_4 - T_3)] + $$
$$[(100 - M_4)\times\ln(T_{max} - T_4)]\rangle,$$

where $LT_{50}$ is the temperature, which create 50 per cent mortality, $T_1 - T_4$ are the experimental test temperatures for sample 1–4, $M_1 - M_4$ are the mortalities in per cent within sample 1–4, $T_{max}$ and $T_{min}$ are the temperatures, by which the mortality is 100 per cent and 0, respectively.

In all cases, where the warmest and coldest test temperatures yielded 0 and 100 per cent mortality, respectively, $T_{max}$ was set to T4 and $T_{min}$ to T1. Where these conditions were not met, it was necessary to estimate an outlying $T_{min}$ or $T_{max}$. Graphs of the mortality as function of test temperature were used to estimate the slope of mortality on temperature. Values of $LT_{50}$ were not estimated if mortality rates for a given test were all above or below 50 per cent. Treatments, for which an $LT_{50}$ value could not be calculated, are in some cases shown in the graphs as the test temperature closest to 50 per cent mortality with an arrow indicating the direction of the true $LT_{50}$.

A measure for the temperature resulting in 50 per cent damage, i.e. $DT_{50}$, was estimated wherever possible. This term comprised mortality as well as deformations and prolonged dormancy. With the chosen test temperatures, damage levels were often too high to give a sound estimate of $DT_{50}$ values. Out of 71 Noble fir $LT_{50}$ values in our dataset, we could only estimate 41 corresponding $DT_{50}$ values. For analysis of the variation among organs, a $\Delta LT_{50}$ value was calculated as the difference between $LT_{50}$ of apical buds and corresponding values in another plant compartment.

### Plant traits, plant compartments and vigour

Frost hardiness was evaluated on damage to apical (leader) bud and sub-apical buds (i.e. the ones forming the next branch whorl). A slight frost

damage resulted in distorted emerging shoots developing from surviving healthy tissues parts such as a group of needle primordia or a lateral bud meristem within otherwise necrotic bud contents. Buds that failed to break normally was inspected visually after cleaving with a scalpel and recorded as either dead (completely necrotic bud content) or apparently dormant. In the latter case, the bud contents were apparently healthy or had a minute necrotic spot at the basis. Frost damage was also evaluated on needles more than 1 year old and on stem base cambium.

Damage to subapical lateral buds was evaluated in percentage of buds present, mostly 2–5. Needle mortality was estimated visually in per cent of total foliage on the tree. Death of the cambium at stem base was evaluated visually by browning of the tissue in one to four tangential bark sections per tree. The responses of the tested organs were quantified in individual plants after bud break (early June) and mortality and damage frequencies were calculated for each sample of nine trees. In cases of doubt, plants were re-assessed in mid-July. Results for apical buds and needles are detailed below, whereas data for lateral buds and cambium are only analysed and presented in relation to those of the apical buds.

During plant response assessment, the overall vigour in terms of extension of new shoots and needles was qualitatively judged at treatment level. Since the objective was to evaluate the effects of acclimation regimes and warm spells, these observations were limited to samples that had only been subjected to the mildest freezing tests and supplementary control samples from the sites. During year 3, current plant mortality was assessed at all acclimation sites throughout the winter.

### Statistical analyses

Statistical analysis was primarily carried out on the raw mortality and damage data, not on the estimated $LT_{50}$ values. We tested differences between treatments and species within test occasions. We could not test differences between test times, because the test temperature levels changed. For the mortality of buds (dead or alive), we used Fisher's exact test, for percentage data on needle mortality Kruskal–Wallis test. When sample sizes were equal and mortality rates developed gradually across the three or four test temperatures, statistical tests were carried out on the pooled data from all test temperature levels. If these conditions were not met, for instance when sample sizes were unbalanced due to pre-test mortality, tests were carried out for each temperature level separately. Comparisons between treatments or species were always pairwise in order to create true ranks between treatments/species. Significant differences in raw data are shown on the $LT_{50}$ graphs by different lettering, based on a 10 per cent two-tailed significance level (e.g. Figures 3–7).

Frost hardiness in subapical bud (year 1 and 2) and in cambium (year 1) was compared with that of the apical buds, the $H_0$ hypothesis being no difference between organs. Although data did not fully meet the statistical requirements, differences in $LT_{50}$ were tested with analysis of variance (general linear model, SAS Institute Inc. 2007) for effects of species, acclimation treatment, warm spells and month (Figures 8 and 9).

The distribution between various types of damage was analysed on raw freezing test data with non-parametric tests (Kruskal–Wallis) – Figure 10.

## Results

### Temperatures at acclimation sites

The acclimation regimes varied with respect to average temperatures as well as temperature fluctuations and roughly followed the intentions that were set out (Figures 1 and 2). The ambient sun-exposed regime A0 outranged all others with respect to weekly fluctuations. Regime A − 5 was on average 3° below A0, and Af 1° below A0.

### Acclimation regimes and frost hardiness of *A. procera*

The controlled temperature chamber set to ambient temperature ($A0_{ctc}$) tended to provide slightly less frost hardiness than the field site A0 but the differences were not statistically significant. This permitted us to directly compare results at A0 with those obtained in controlled temperature chambers.
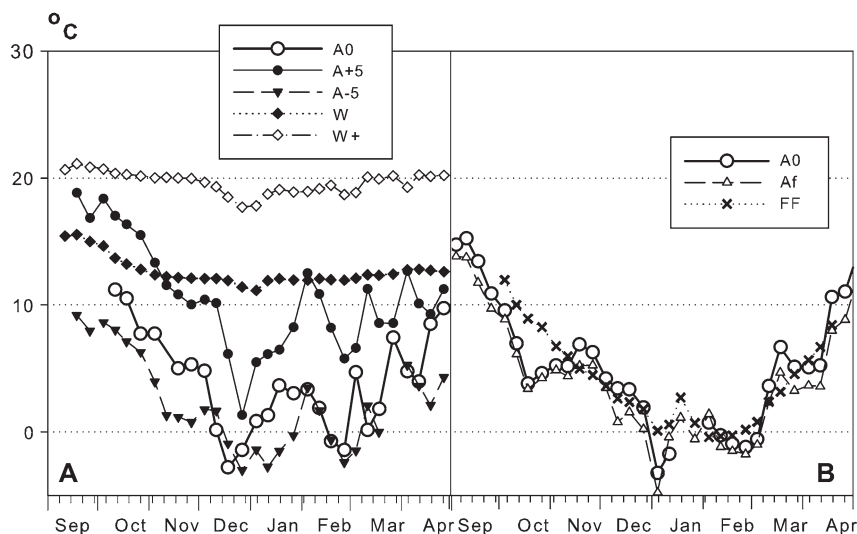
*Figure 1.* Weekly average temperatures in acclimation regimes used in this study (cf. Table 1). Above: means of test years. Below: A0 and Af from year 2 and FF from year 3. Major ticks on abscissa indicate month intervals and minor ticks week intervals.
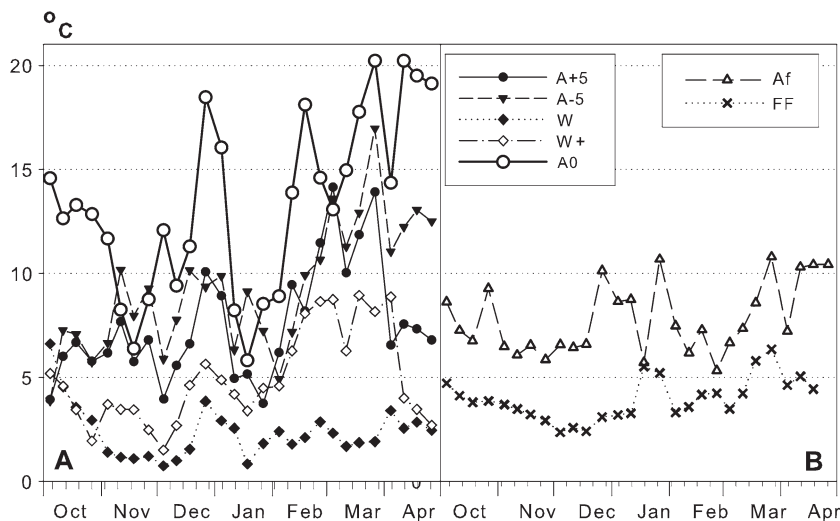


*Figure 2.* Weekly range of temperatures (weekly max. to weekly min.) in acclimation regimes. Otherwise as in Figure 1.

Frost hardiness increased during autumn and peaked from January to March at ambient temperatures but showed some year-to-year variation. Generally, lowered temperatures (A − 5) during the first test year resulted in greater hard-ening during late autumn but less frost hardiness (or earlier dehardening) during spring, compared with ambient temperature (Figure 3). This pat-tern was apparent both in respect to apical bud and needle mortality, with the crossing of curves
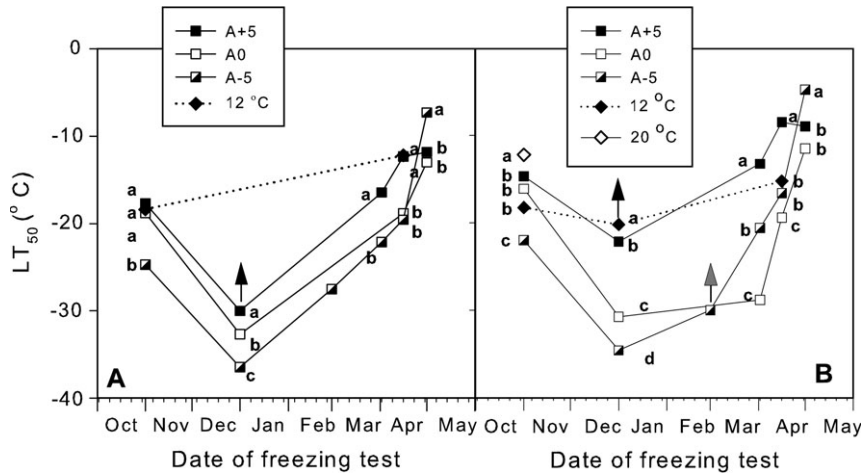
*Figure 3*. Seasonal development in frost hardiness ($LT_{50}$) in year 1 in *Abies procera* as a function of temperature acclimation regimes (cf. Table 1). (A) apical bud mortality and (B) needle death. Points with different lettering indicate that freezing tests differed at $P < 0.10$ level. Arrows indicate insufficiently determined $LT_{50}$ values and point in the direction of the true value (cf. text for calculation of $LT_{50}$).

occurring around mid-February to early March. An overall increase in temperatures (A + 5) resulted in less deep frost hardiness than the A0 control (Figure 3). In year 2, frost hardiness was on the whole less deep and there was no consistent differentiation between acclimation regimes A0, A + 5 and A − 5. The sheltered outdoor acclimation regime (Af) consistently showed a deeper frost hardiness, both in respect to apical bud and needle mortality, than the A0 regime throughout winter, but the difference was not statistically significant (data not shown).

Trees at constant and 'warm' acclimation temperature (12°C), and thus deprived of most seasonal temperature cues, early in autumn developed a rather low $LT_{50}$ value of −18°C (Figure 3). Even the 20°C acclimation regime induced an $LT_{50}$ value of −12°C during autumn (Figure 3B). Cold hardiness, however, did not increase further during winter as we found in the other regimes.

### Comparison with other species

Development of frost hardiness in the two species of *Abies* resembled each other in year 1, especially in bud mortality (Figure 4), but *A. procera* showed significantly poorer frost hardiness than

*A. nordmanniana* throughout the seasons in year 2 (data not shown). Except in autumn and late spring, *P. sitchensis* developed a slightly deeper frost resistance than *Abies* spp., and *P. abies* clearly developed the deepest frost hardiness in both test years (Figure 4).

### Warm-spell treatments

#### Autumn
*Abies procera* was subjected to autumn warm-spell treatments in year 2 and 3, *A. nordmanniana* only in year 3 (Figure 5). During year 2, *A. procera* was treated in the cool acclimation site (A − 5) before warm-spell treatment (eWS Table 2). In year 3, the acclimation before and after warm spell was set to a 30-year average temperature trend (FF), in order to avoid experimental errors due to natural extremes. Under these conditions, warm spells did not trigger a dehardening in *A. procera*; actually after the October warm spell, a significantly stronger hardening was found (Figure 5A, B, E, F). In contrast, *A. nordmanniana* reacted to a November warm-spell treatment with severe dehardening (Figure 5C, D). Re-hardening in this species was fast in the buds, less so in the needles.
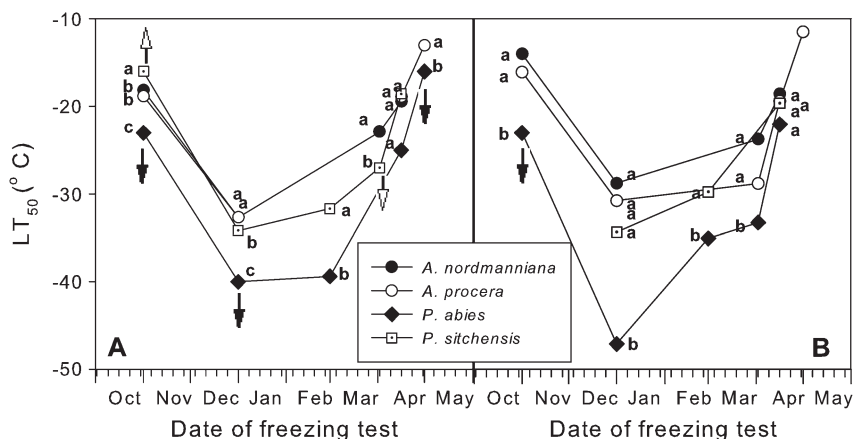
*Figure 4*. Tree species differences in frost hardiness ($LT_{50}$) in year 1 after acclimation at site A0 (cf. Table 1). (A) Apical bud mortality and (B) needle death. Otherwise as in Figure 3.

In year 2, the *A. procera* subjected to the eWSNov treatment experienced severe natural frost on the acclimation site on the night before freezing test on 1 January; hence, the resulting data point was discarded.

### Late winter/spring

Ten days of warm-spell treatment in late winter/ early spring (Table 2) resulted in an immediate, considerable loss of hardiness, which was still significant in the beginning of April, 2–6 weeks after the warm-spell exposure, and in both apical bud and needles (Figure 6). In *A. procera* and *P. abies*, the dehardening curves of warm-spell treated and control samples would converge and eventually meet, several weeks after treatment, indicating a complete recovery to seasonal hardiness in late April (Figure 6A, B, E, F). Recovery appeared to be much slower or not occurring at all in *A. nordmanniana* and *P. sitchensis* (Figure 6C, D, G, H).

Nine-day treatment of *A. procera* in February tended to decrease apical bud and needle frost hardiness significantly with ~4 weeks' delay (Figure 7A, B). In March, the effect was more rapid. In both cases, a recovery took place in subsequent weeks. Both reactions appeared particularly strong in the needles. A 6-day exposure in February tended to have a hardening effect, while in March the effect tended to cause dehardening (Figure 7B). Short exposures (3–6 days) had no significant influence.

### Comparison of organs

The subapical lateral buds (whorl buds) had higher $LT_{50}$ values, and thus poorer frost resistance, than apical buds, the difference in $LT_{50}$ (i.e. $\Delta LT_{50}$) being on average 4°C for both species of *Abies* across all data, but this varied seasonally. Indicative analysis of variance on the $LT_{50}$ values showed no significant effects of year, acclimation regimes and warm-spell treatments and no interaction. Thus, all further analysis was restricted to the A0 material. In the two species of *Abies*, $\Delta LT_{50}$ for lateral buds varied with month in a highly significant manner, being greatest in the coldest months ($P < 0.0001$, $R^2 = 0.35$, Figure 8). The two species in *Picea* had a significantly smaller $\Delta LT_{50}$ between apical and lateral buds than the *Abies* species (Data not shown).

$\Delta LT_{50}$ between apical bud and cambium showed no effects of treatment and warm spells; all further analysis only included the A0 material, comprising 12 data pairs. The stem cambium generally had lower $LT_{50}$ values (deeper frost hardiness) than apical buds; $\Delta LT_{50}$ varied with species and time of year, but with no correlation to frost hardiness level (Figure 9). Differences between species were considerable ($P = 0.05$). The two north-west American species *A. procera* and *P. sitchensis* seemed to be characterized by small $\Delta LT_{50}$ that remained fairly stable over the year, in contrast to the two Eurasian species *A. nordmanniana* and *P. abies*. In *Abies*, the cambium tended to deharden more slowly in spring than the apical buds (one-way ANOVA, $P = 0.01$).
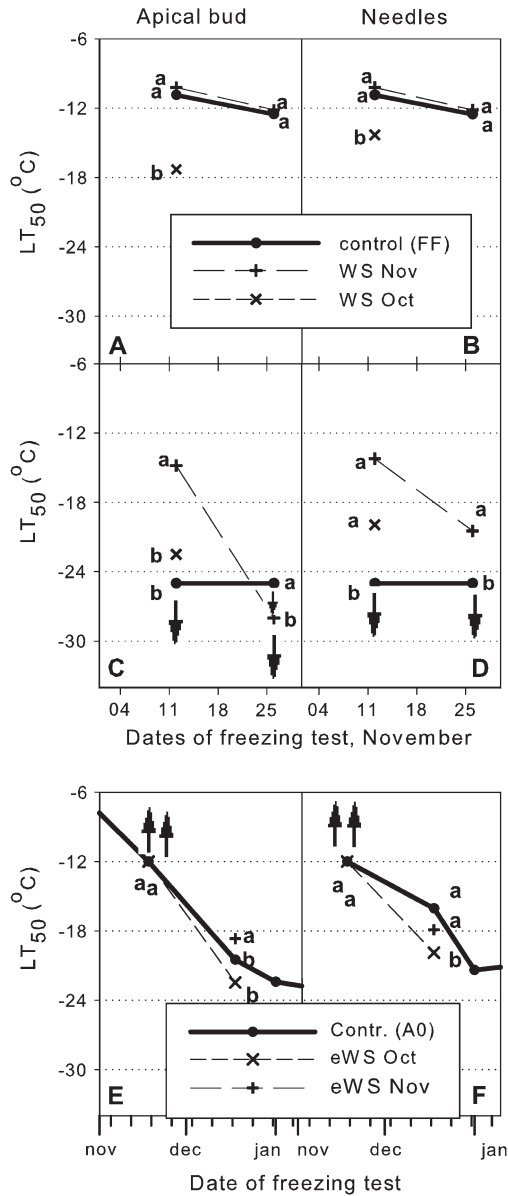
*Figure 5.* Dehardening and recovery after 14–15 days warm-spell treatments during autumn (cf. Table 2), with acclimation at A0 (year 2) and FF (year 3). October and November treatments ending 5/11 and 12/12 (year 2) and 1/11 and 12/11 (year 3). (A, C and E) Apical bud mortality and (B, D and F) needle death. Abscissa in (A–D): dates in November. Points with different lettering indicate that freezing tests differed at *P* < 0.10 level. A–B, E–F: **A. procera**, y 2 and 3, resp., C–D: **A. nordmanniana.**
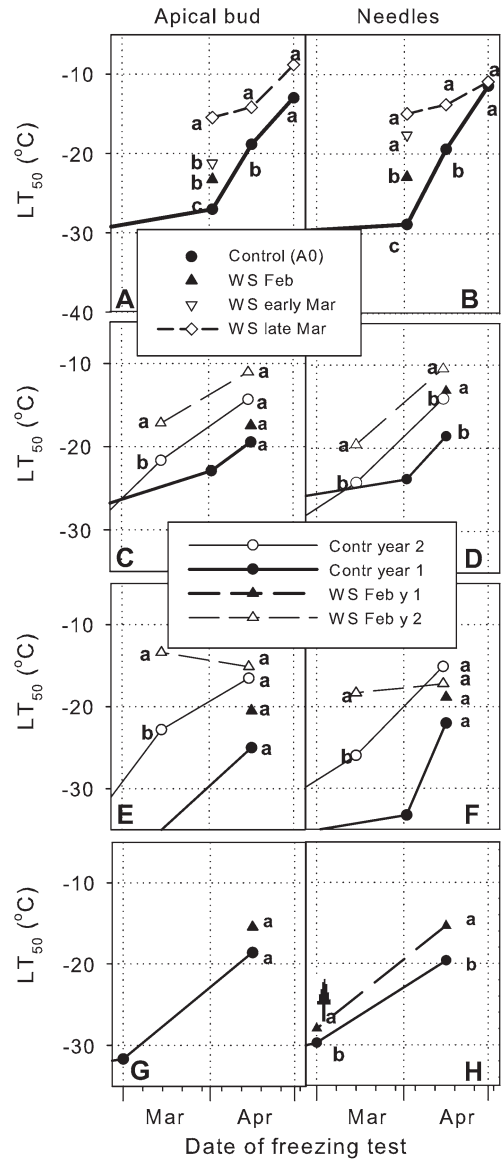
*Figure 6.* Responses to 9–10 days warm-spell treatments in spring. *Abies procera* data from year 1, otherwise data from year 1 and 2. February treatments ended at 21/2, early March at 12/3, late March at 30/3, in year 1. February treatment ended at 11/2 in year 2. (A, C, E and G) Apical bud mortality and (B, D, F, and H) needle death. Points with different lettering indicate that freezing tests differed at *P* < 0.10 level. A–B: **A. procera**, C–D: **A. nordmanniana,** E–F: **Picea abies,** G–H: **P. sitchensis.**

*Figure 8.* Frost hardiness in lateral buds compared with apical buds: difference in $LT_{50}$ values ($\Delta LT_{50}$) over the season (A) and in relation to $LT_{50}$ of apical bud (B). Monthly adjusted means for the $\Delta LT_{50}$ between bud types based on pooled data of the two *Abies* species. Data from the A0 acclimation, year 1 and 2.
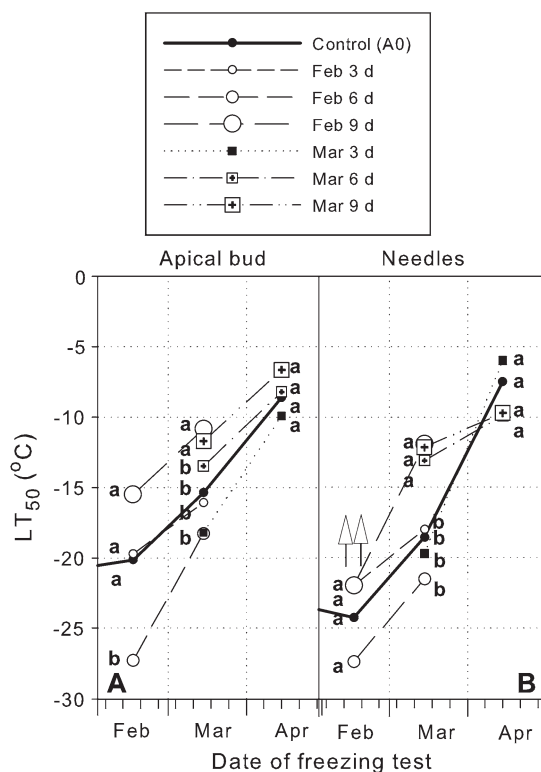
*Figure 7.* Warm-spell treatments of varying length (3, 6 and 9 days) in spring, *Abies procera*, year 2. February treatments ending at 11/2 and March treatments at 11/3. (A) Apical bud mortality and (B) needle death. Points with different lettering indicate that freezing tests differed at *P* < 0.10 level.

### Sub-lethal bud damage

The $DT_{50}$ of buds – comprising all types of frost damage, including mortality – was on average 2.5°C higher than the $LT_{50}$ values in *A. procera*. However, the true difference would probably be considerably larger because $DT_{50}$ values that could be estimated were biased towards greatest similarity to corresponding $LT_{50}$ values (cf. Materials and methods).

Bud mortality, of course, generally declined with increasing test temperatures (Figure 10). Apparent dormancy in buds was the prevalent type of damage at the mildest test temperatures (year- and species-wise Kruskal–Wallis tests). Only at tests in October, apparent bud dormancy occurred at all freezing temperatures (October data not included in Figure 10).
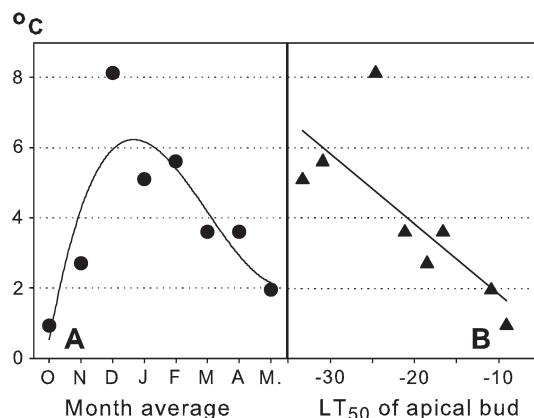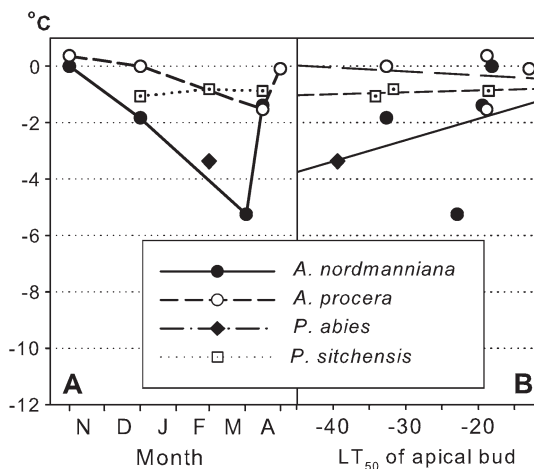


*Figure 9.* Frost hardiness in cambium compared with apical buds. Difference in $LT_{50}$ values ($\Delta LT_{50}$) over the season (A) and in relation to $LT_{50}$ of apical bud (B), in different species. Monthly adjusted means for the $\Delta LT_{50}$. Data from the A0 acclimation, year 1.

The damage pattern varied significantly over the year in all species. A clear seasonal trend was only found in the two north-west American species *A. procera* (Figure 11) and *P. sitchensis*, where apparent bud dormancy occurred as a particularly common damage type in October and in
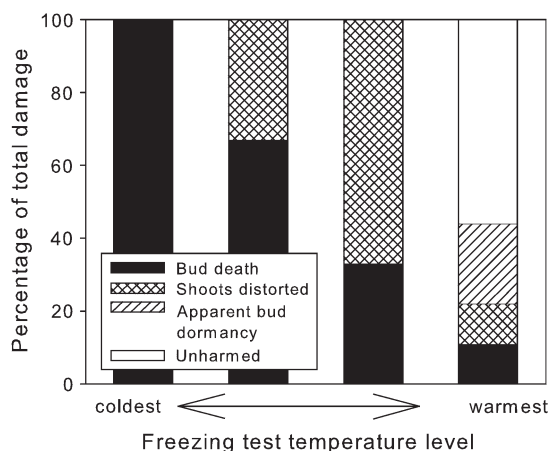
*Figure 10.* Sublethal damage: three types of bud disturbances in response to the four levels of the freezing tests, from coldest (left) to warmest (right). Data from the *Abies procera* frost test on 18. Dec, test year 1, material from acclimation regime Af.
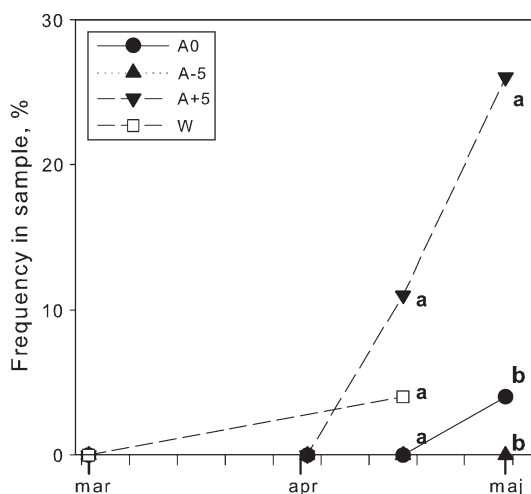


*Figure 11.* Frequency of apparent dormancy among the sublethal types of freezing damage occurring to apical buds, at various acclimation regimes. *Abies procera*, year 1. Means of data from all freezing test temperatures (*n* > 36). Point with different lettering indicate differences at *P* < 0.10 level.

spring. 'Warm' acclimation regimes (A + 5 and presumably W, Figure 11) and, to some extent, warm-spell treatments (data not shown) resulted in higher frequency of apparent dormant buds

than outdoor regimes and the 'cold' A − 5 acclimation regime, when freezing tested in spring.

### Vitality of plants after acclimation

The impact of acclimation regime on general vigour in *A. procera* was obvious to a visual and qualitative inspection in the following summer. A cold regime (A − 5) induced red needles in the uppermost shoots of the plants already during the winter acclimation and resulted in short shoots after flushing. Plants at strongly fluctuating temperatures (A0) had no reddening of the mature needles, but the shoots unfolding in the next growth season were short. A much higher vitality was found in the 'mild' acclimation regimes A + 5 and above all in regimes with reduced temperature fluctuation (Af and FF). This was expressed in healthy and green mature needles and long-leaved and vigourous new shoots. Vitality was intermediate after a winter at 12°C (W) and comparatively poor after acclimation at 20°C (W+). Similar tendencies were found in *A. nordmanniana* (year 3).

These qualitative observations were largely confirmed by the current mortality (i.e deaths among trees not subjected to freezing tests) within samples as monitored throughout the winter in year 3 (Figure 12).

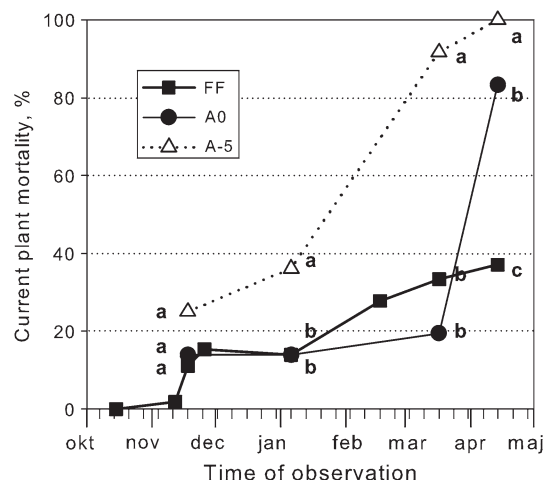

*Figure 12.* Plant mortality in *Abies procera* during winter of year 3 at three acclimation regimes. The sudden mortality increase at site A0 in April is due to three warm weeks in March and a sudden cold night (−10°C) at April 8.

## Discussion

### Mechanisms of acclimation

It is well established that decreasing day length and decreasing temperatures induce frost hardening (Irving and Lanphear, 1967; Owston and Kozlowski, 1981; Sakai and Larcher, 1987; Kozlowski and Pallardy, 2002; Colombo et al., 2003), but it requires an experimental setup to distinguish between these two environmental cues. Thus, for *Pinus sylvestris*, Smit-Spinks et al. (1985) found a cold hardiness down to −40 on short days and cold temperatures, whereas cold hardiness only reached −22 on short days alone. Our results from the warm acclimation sites (12 and 20°C, Figure 3) show that day length alone induced a fairly high frost resistance in early November ($LT_{50}$ of −18 and −12°C, respectively). This in spite of that *A. procera* is a comparatively southern species would normally be less dependent on day length than boreal species. However, decreasing temperatures were necessary to develop deeper frost hardiness during mid-winter. This complies with the models of a multi-stage hardening process as discussed by Kozlowski and Pallardy (2002), Sakai and Larcher (1987) and Glerum (1976, 1985). However, very low temperatures as a third trigger for deep hardening could not be confirmed for *A. procera*: the strongly fluctuating temperatures at acclimation site A0 did not induce greater frost hardiness than the sheltered forest site (Af), although the plants at A0 were subjected to lower minimum temperatures (Figures 1 and 2). In fact, the opposite tendency was found. This might be explained by energically costly adjustment of trees to constantly fluctuating temperatures (see discussion below).

Nevertheless, temperature trends showed clear and important effects on hardening and dehardening throughout the winter season. Constantly elevated temperatures (A + 5) reduced frost resistance and constantly lowered temperatures (A − 5) increased frost hardiness in the early winter season (Figure 3) – see also (Redfern and Cannell 1982) Redfern and Hendry (2002). The negative effect from excessive cold on hardiness in late winter of year 1 and during year 2 might also be ascribed to depletion of carbohydrates.

### Acclimation and carbohydrate balance

Establishment of cold hardiness is closely linked to nutrient status and general vigour (Sakai, 1978). A decrease in stem dry weight during autumn suggests that hardening is an energy requiring process that depletes storage reserves (Bigras et al., 1996). Plants with low carbohydrate content tend to become less cold hardened (Sakai and Larcher, 1987), whereas high summer temperatures (which would stimulate photosynthate production) tend to strengthen cold hardiness (Balduman et al., 1999).

Our observations suggest that continuously intense cold or frequent and large temperature fluctuations require the consumption of significant parts of the carbohydrate reserves in small trees. This conclusion is partly based on flushing vigour, general tree mortality (text and Figure 12) and development of frost hardiness and dehardening under various acclimation regimes (text and Figure 3). It seems that temperature fluctuations have a stronger negative effect than low temperatures *per se*; the colder but more stable Af site appeared to perform better than the non-sheltered A0 site. Trees in the A-5 site had poor vigour that could be due to both stronger cold and large fluctuations.

The effects of cold exposure and fluctuating temperatures, in particulary, were pronounced in *A. procera* and to a lesser degree in the other species.

### Warm spells

The significant effect of temperature trends at all times outside the growing season is furthermore corroborated by the warm-spell treatments, carried out in October/November and February/March (Figures 5–7). Little literature was found on the effect of warm spells on frost hardiness apart from the general discussion by Sakai and Larcher (1987) and Bigras et al. (2001), finding that frost hardiness may fluctuate in response to temperature fluctuations during winter. Both Sakai and Larcher (1987) and Bigras et al. (2001) seem to link the ability to harden and deharden with the stage and depth of dormancy: 'At the onset of winter, woody plants react more strongly to hardening than dehardening stimuli'. On the other hand, still according to Sakai and Larcher

(1987), trees are more easily dehardened and less easily re-hardened in late winter, at the post-dormancy stage. This general pattern is not entirely supported by our results that seem to uncover considerable specific differences: Although *A. procera* did not deharden in response to a warm spell in autumn, *A. nordmanniana* did so to a serious extent (Figure 5A, B *vs* C, D). Both species reacted with dehardening in late winter/early spring, but rehardening only occurred in *A. procera* (Figure 6). Thus, in autumn, *A. procera* confirmed the expectation of Sakai and Larcher (1987) but in spring contradicted it. The opposite was found for *A. nordmanniana*. Bigras *et al.* (2001) also refer to species differences in reactions to warm spells.

Our experiments indicated that the component of frost hardiness brought about by low-temperature stimuli is not very stable in *A. procera*. Nine to ten days of warm-spell treatment broke down temperature-induced hardening. Furthermore, our results suggested that the photoperiod-induced hardiness was also affected, i.e., hardiness decreased below the level achieved by day length alone, which is in line with the findings of Zhang *et al.* (2003). It thus seems that temperature fluctuations can override the control of day length in *A. procera*. This seems reasonable, considering the assumed lower importance of photoperiod than of temperatures as environmental cues in this species (cf. Introduction).

Interestingly, length of warm spells in early spring influenced the result considerably: A period of 3 days of warming has no significant effect, 6-day treatment sometimes improved, sometimes decreased the frost hardiness, whereas 9–10 days of warming resulted in an often dramatic decrease of frost hardiness. The hardening effect of 6 days warming might be related to a strongly needed carbohydrate build up during late winter. On the other hand, more than 6 days warm spell appear to set forth dehardening processes that are reversed very slowly when the trees are returned to the cold. From this, one may gather that the timing and length of warm weather episodes in nature are quite critical to how they affect hardiness.

### Differences between species

Frost hardiness ranking of species was roughly: *A. procera*, *A. nordmanniana*, *P. sitchensis* and *P. abies*, with *A. procera* as the most sensitive. However, the performance of *A. procera* varied strongly with year and we attributed this to provenance differences (cf. Rehfeldt, 1980; Larsen, 1986; Nielsen *et al.*, 1996). *Picea sitchensis* deviated from the above ranking in autumn and late spring (Figure 4), in agreement with practical experience in Northern Europe with provenances originating in Oregon or Washington. *Picea abies* is usually found to be highly frost resistant (Lindström and Nyström, 1987; Nielsen *et al.*, 1989).

### Plant compartments and types of damage

Frost hardiness ranking between plant compartments was generally stem cambium, apical bud and needles and subapical buds. This supports Nielsen *et al.* (1996), although the ranking in organ hardiness may differ in other tree species (Sakai and Larcher, 1987). It makes sense in evolutionary terms that the stem cambium and the apical bud should be the most resistant structures, as they are hard to replace and most important for the survival of the tree. In cases of severe frost damage in *P. sitchensis*, re-growth from the base of the stem be observed. This regeneration pattern might also relate to the exceptionally high root frost resistance of this species (McKay, H. and C.C.N. Nielsen unpublished data).

It is noteworthy that in the species of *Abies*, we found much stronger frost protection in apical than in subapical buds: in these species, a strong shoot dimorphism makes the apical bud unique and more difficult to replace than in the species of *Picea* (e.g. Veierskov *et al.*, 2007). The apical bud is also strongly protected physically by the whorl of tightly encircling subapical buds; the bud whorl in *Picea*, in comparison, is more widely spaced, allowing the apical bud to be fully exposed. The rather considerably less frost hardiness of lateral buds in the *Abies* species corresponds with the well-known high mortality of lateral buds in Denmark that often severely disturbs crown symmetry. Some *A. procera* provenances that have lower frost resistance than *A. nordmanniana* should in general suffer relatively more from the lowered hardiness of lateral buds. This is confirmed by field observation.

In contrast to the *Picea* species, whose buds tend to either die or survive unharmed (Nielsen

*et al.*, 1989), several damage types were frequent in *A. procera* (Figure 10) and also occasionally found in *A. nordmanniana*. Except in autumn, the distribution of damage types across test temperatures indicated that the prolonged 'apparent dormancy' with green bud content could be induced by comparably mild frost levels, whereas 'distorted shoots' developed from partially surviving bud material at somewhat lower temperatures. Deeper frost levels totally killed buds. The apparent dormancy is most likely a sublethal damage to bud cells, but in many cases invisible in cross-sections or with tiny necrotic spots. The stem pith below distorted shoots sometimes showed browned tissue, but a loosening of the pith from the bud basis, as described elsewhere (Lewis and Dowding, 1924) was not found.

### Climate change and geographic variation

Two central papers on climate change and frost (Cannell and Smith, 1986; Hänninen *et al.*, 2001) do not deal specifically with warm-spell problems. The practical experience with cultivation of *A. procera* in the mild-climate of Southern Ireland and Britain is generally good, which indicates that the expected elevated future temperatures (IPCC, 2007) will not present a problem to *A. procera* winter physiology. Reduced frost hardiness under mild winters, as in acclimation regime W and A + 5 (Figure 3), needs not constitute a problem as long as the climate follows a stable seasonal pattern. The warm-spell experiments suggest, however, that problems may arise from unseasonable and unstable climate conditions. It is thus of larger concern that IPCC (2007) forecasts an increasing frequency of extreme climatic events, including warm spells. This problem is likely to be particularly severe in those geographic areas where different climate systems intermingle and change dominance. The Northern Central European (Sweden, Denmark and Germany) is alternatingly under oceanic influence from the Atlantic and continental influence from the European mainland and will most likely experience increasing climatic extremes. This could mean increased bud mortality in cultures of *A. procera*. Besides temperature fluctuations, a problem could also arise with rising atmospheric $CO_2$ that could delay frost hardening in autumn, as suggested in other tree species (Loveys *et al.*, 2006).

Snow cover insulates seedlings and reduces temperature fluctuation (Sakai, 1966). The results presented here predicts that in colder geographic areas young *A. procera* are likely to grow poorly if not covered by snow during winter, the problems being due to frost damage as well as low carbohydrate accumulation combined with high-energy costs during temperature re-adjustments. This may be the reason for the southern natural distribution of this species.

North of its natural habitat in Canadian British Columbia, the utilization of *A. procera* is mainly restricted by low winter temperatures and insufficient growth season moisture, according to Ying (1992) and Xie and Ying (1994): mortality and winter frost damage increased severely from the coast towards the sub-continental inland and height growth decreased. This suggests that the species would be culturable further north along the coast than at inland localities. In the mild oceanic climate of Scotland, *A. procera* grows well as far north as 57° N, whereas frequent bud problems occur at this latitude in the subcontinental conditions of Denmark. *Abies procera* is to our knowledge not used in continental countries further east in Europe. Thus, Denmark and South Sweden seem to form the eastern limit for cultivation of this species in Europe. However, 'insufficient growing-season soil moisture' as second limiting factor (Xie and Ying, 1994) cannot be confirmed by European experience, where *A. procera* perform well on dry sandy sites with only 300 mm precipitation during the growing season and where the main problems for establishment are poor root growth potential and low root desiccation resistance (McKay *et al.*, 1997).

### Microclimate

In the subtropics, the topographic aspect has major influence on plant growth conditions during winter (Saseendran *et al.*, 1993), whereas in arctic/subarctic areas the aspect has more influence during the summer (Yanagihara *et al.*, 2000). In temperate regions, aspect effects in a sloping growing site are most pronounced during autumn and spring (Macyk *et al.*, 1978; Stephenson, 1982). The temperature trend which is largely responsible for hardening and dehardening in most species (Kozlowski and Pallardy, 2002) will induce later hardening in autumn

and faster dehardening in late winter on south-facing slopes at temperate latitudes. Because *A. procera* developed severe non-lethal damage at temperatures considerably higher than $LT_{50}$ (Figure 10), and because such bud disturbances were more frequent at mild acclimation sites (Figure 11), south-facing slopes are likely to predispose *A. procera* towards bud disturbances. This might explain why crown shape quality is very site dependent in Denmark. South-facing slopes are also subjected to higher daily temperature fluctuations (Sakai, 1966, 1970), which seem to diminish the carbohydrate stores and reduce frost resistance of *A. procera* seedlings (comparison of site A0 and Af).

The forest-sheltered Af site in our study had generally lower temperature fluctuations (Figure 2) than the A0 site, and during cold nights the temperature was generally 2–4° warmer than the A0 site. This is in agreement with other studies of shelterwood (Langwall and Örlander, 2001). These findings strongly indicate that north-facing slopes and sheltered sites (shelterwoods, strip systems) induce a better frost resistance and vigour in *A. procera*.

## Conclusions

While *A. procera* develops undisturbed crowns in geographic areas with either constantly mild oceanic climate or continuous winter snow cover, various disturbances of the terminal apical bud as well as lateral bud mortality occur frequently and constitutes an extensive problem in central Northern Europe. This paper shows that large temperature fluctuations as well as unstable climate conditions with intermittent warm periods during autumn and late winter tend to have a negative effect on frost hardening, dehardening and, probably, spring carbohydrate stores in seedlings. The ongoing climate change is likely to enhance these problems in the future in areas with unstable climatic influence.

In areas with climatic conditions that are unfavourable for *A. procera*, it is recommended to grow this species in sun-sheltered environments in order to reduce the daily temperature fluctuations and the effects of intermittent warm periods during spring and autumn. Favourable sites may be on north-facing slopes and in the shelter of older trees (e.g. east–west stripwise regeneration). This does not necessarily contradict the observation of Franklin (1982) that *A. procera* performs best on south-facing slopes in the natural habitat of the Cascades. Under these natural conditions, the trees have a shorter growing season, with more predictable temperatures and a long winter snow cover.

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