

*resinosa* did amplify homologous sites in distantly related species, suggesting that the targeted region was highly conserved in a broader range of species within the genus *Pinus*.

Although the simultaneous amplification of multi-copy loci can sometimes be difficult to analyse when the bands overlap, there are advantages when their inheritance patterns can be discerned. Firstly, many alleles can be scored per individuals from one set of specific primers. A second advantage occurs when the multiple copies are at the same locus. This increases the chance that the locus will show polymorphism and can be used for genetic analysis. Lastly, unlinked multiple loci can be assessed simultaneously, providing a natural multiplex for mapping, parentage testing, and other analyses (FISHER et al., 1998).

Microsatellite markers are abundant in the genomes of nearly all eukaryotes. But the development of SSR markers is technically demanding, expensive and time consuming compared to other genetic markers. The use of DNA sequence similarities between taxa should contribute to leverage SSR and related markers development efforts. In the present study, the informative primer pair was derived from *P. resinosa* sequence around the SSR region. This pair was useful for genotyping in other species analyzed. If suitable numbers of informative SSR and related marker primer pairs developed from one conifer species could be used for genotyping in related species, then overall SSR marker development costs would be available, and SSR marker analysis could be more widely incorporated in many conifers genetics programs (ECHT et al., 1999). The polymorphic loci observed among species could be useful as species-specific markers if additional testing on a broader sampling of individuals in each species confirm these putative specific markers. The data set generated can be of use in phylogenetic studies and the identification of interspecies hybrids.

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# Variation in Chilling Requirements for Completing Bud Rest Between Provenances of Norway Spruce

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## Abstract

Winter dormancy in forest trees starts with a rest stage, during which buds remain inactive even if they are exposed to growth-promoting conditions, and is followed by quiescence. The transition from rest to quiescence requires chilling. The amount of chilling needed to complete the rest phase was tested in different provenances of Norway spruce (*Picea abies* (L.)

Karst.). One-year-old seedlings from eight provenances were grown in a climate-chamber experiment, and from ten provenances (with one Sitka spruce provenance, for comparison) in a nursery experiment. The rest status of these seedlings after exposure to chilling for various durations was tested by measuring the time they took to break bud after transfer to growth-promoting conditions. In the nursery experiment, the chilling requirement was high in provenances from Denmark, Germany and Belorussia, and low in provenances from northern Sweden and Russia. Provenances from southern Sweden and Norway had intermediate chilling requirements. The Sitka spruce provenance had a very high chilling requirement. A high chilling requirement suggests better adaptation to maritime conditions and warm winters. However, absolute differences between provenances were small. The chilling requirement was

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mostly fulfilled in November–December. In the climate chamber experiment the chilling requirement could not be determined, because the rest phase had started to break before the chilling treatment was applied. The northern Swedish provenance consistently showed the lowest temperature sum requirement for budburst while the southern German provenance showed the highest temperature sum requirement in most treatments.

*Key words:* budburst, chilling, dormancy, *Picea abies*, phenology, rest completion, rest break.

## Introduction

Dormancy is the part of the annual growth cycle of trees during which no visible growth occurs (ROMBERGER, 1963; HÄNNINEN, 1990). In trees from the temperate zones dormancy is commonly divided into two stages: the rest phase, during which the buds remain inactive even if they are exposed to growth-promoting conditions, and the subsequent quiescent phase, during which growth can resume whenever conditions become favorable (ROMBERGER, 1963; SARVAS, 1974). The transition from rest to quiescence is promoted by chilling, i.e. exposure to cold temperatures, usually between 0 and +10°C (NIENSTAEDT, 1967; WORRALL and MERGEN, 1967; SARVAS, 1974; FUCHIGAMI et al., 1982). A lack of chilling may sometimes be compensated for by long days (e.g. WORRALL and MERGEN, 1967; CAMPBELL and SUGANO, 1975; DORMLING et al., 1968; QAMARUDDIN et al., 1995). Furthermore, long nights may also release dormancy, according to studies under controlled conditions (QAMARUDDIN et al., 1993). However, in boreal conifers in field conditions, chilling seems to be the main factor promoting rest completion (WORRALL and MERGEN, 1967). Once the rest period has been completed, growth can commence if the tree is exposed to warm temperatures. Rest may, therefore, be a strategy that prevents the resumption of growth if temperature increases too soon after the initiation of dormancy (i.e. in the same autumn or during the following winter).

The suitability of trees for use in forest cultivation is highly dependent on the synchronisation of their inherent growth rhythms to the local site conditions. Hence, much effort has been invested in characterizing the genetic variation in growth rhythm traits in order to identify optimal varieties for cultivation in specific areas. Most studies on conifers in the temperate zones have focused on the timing of budburst in the spring and growth cessation in the autumn, because these characters are strongly related to the risk of frost damage (see review in AITKEN and HANNERZ, 2001). However, few studies have considered variations in chilling requirements, a factor that may be important for the adaptation of trees to different winter conditions.

Trees that grow in a maritime climate, e.g. Sitka spruce (*Picea sitchensis* (Bong.) Carr.), usually have high chilling requirements, which ensure that the trees remain inactive and hardy even during winter periods with mild temperatures (CANNELL and SMITH, 1983). For instance, in Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), Scots pine (*Pinus sylvestris* L.) and silver birch (*Betula pendula* Roth) it has been shown that coastal provenances tend to have higher chilling requirements than continental provenances (CAMPBELL and SUGANO, 1979; LEINONEN, 1996).

No corresponding studies have been reported for Norway spruce (*Picea abies* (L.) Karst.). Norway spruce survived the last glaciation in refuges in western Russia and Eastern Europe, from where it spread north and west (HUNTLEY and BIRKS, 1983). The limits of the present natural distribution of Norway spruce coincide well with the isotherm for –2°C, indicating that intolerance of mild winter weather may be one of

the main constraints for the survival and reproduction of Norway spruce (DAHL, 1990). The species has also been widely planted outside its natural range, including areas with maritime climates and mild winters in Great Britain, Denmark and the western coasts of Sweden and Norway. However, damage to needles and shoots during mild winters is common in these areas, e.g. in Great Britain (TRANQUILLINI, 1982), Denmark (RAVENSBECK, 1991), Norway (DIETRICHSON, 1993) and Sweden (HANNERZ, 1994).

Climatic warming is likely to increase winter temperatures, and thus may affect winter chilling, spring warming and the risk of damage to trees. Several studies have focused on the effects of budburst date and the potential risk of frost damage as a result of climate warming (MURRAY et al., 1983; CANNELL and SMITH, 1986; DIETRICHSON, 1993; HÄNNINEN, 1995; KRAMER, 1995). It is essential to include chilling functions in these models. Therefore, predictions of the trees' adaptability to climatic warming and cultivation in maritime areas require knowledge of how chilling requirement varies between different seed sources.

In this study we compared the chilling requirements for rest completion in 1-year-old seedlings of different provenances of Norway spruce. The provenances were also compared with Sitka spruce, a species known to have high chilling requirement. The hypothesis was that provenances from more maritime climates in southern Scandinavia and Western Europe would have higher chilling requirements than provenances from continental climates in Russia and northern Scandinavia.

## Material and Methods

### Overall design

The study consisted of two parallel experiments: one in a nursery and one in a controlled climate chamber in the phytotron at the Swedish University of Agricultural Sciences in Uppsala. Seedlings were sown and cultivated under standard conditions during the first growing season. After dormancy had been induced through long-night treatment, the seedlings were subjected to chilling at a constant temperature of +3°C in the phytotron, while the seedlings in the nursery were exposed to natural outdoor chilling. After various durations of chilling exposure, samples of seedlings were transferred to growth-promoting conditions at +20°C and the number of days they took to reach budburst was recorded. The relationship between the duration of chilling and the temperature sum needed to reach budburst was then analyzed.

### Seed sources

In total 11 different seed sources were included in the study (Fig. 1, Table 1). Three of these were only used in the nursery study, while the other eight were used in both experiments. The seed sources were chosen to represent a wide range of origins in order to cover a broad anticipated range of reactions to the treatments. The Russian sources (1 and 2) and the northern Swedish sources (4 and 10) were chosen to represent material from a typical northern, continental type of response. The Danish seed orchards (7 and 9), and the stand seed from Germany (6) were expected to display more maritime response. The other seed sources were expected to be intermediate in this respect. The Sitka spruce seed source was included in the nursery experiment to represent material with an extreme maritime response, with high presumed chilling requirements.

### Nursery experiment

The seeds were sown on 3–4 April 2000 in peat-filled containers (Hiko V93, 93 cm<sup>3</sup>, 526 seedlings m<sup>-2</sup>) in a greenhouse at

SkogForsk's nursery in Brunsberg (59°37'N, 12°59'E, 75 m a.s.l.). Fertilization started in the first week in May. A full fertilizer (N, P, K and micronutrients) was applied 2–3 times per week, the amount being adjusted to maintain the conductivity of the peat's leachate at 1.5 mS. Fertilization was stopped in early September. The seedlings were kept in the greenhouse under natural light conditions over the summer and a long-night treatment with 16 h dark periods was applied from 1 August until 21 August, when the seedlings were moved outdoors. The seedlings were then kept outdoors for natural chilling treatment until they were transferred indoors to growth-stimulating conditions.

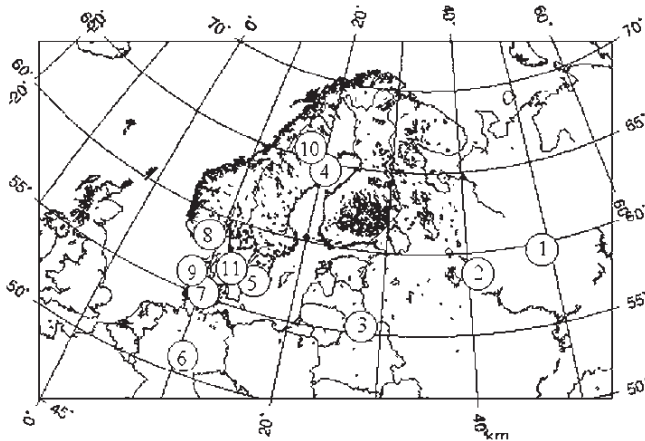


Figure 1. – Geographic location of seed sources used in the experiments. Nos. 1 to 8 were used in both the phytotron and the nursery, nos. 9 to 11 only in the nursery. Nos. 1 to 10 are Norway spruce provenances, no. 11 is the Sitka spruce seed orchard.

Table 1. – Geographical origins of the seed sources.

| ID. | Provenance                            | Latitude N | Longitude E | Elevation m a.s.l. |
|-----|---------------------------------------|------------|-------------|--------------------|
| 1   | Kirov, Russia                         | 59°37'     | 49°05'      | 250                |
| 2   | Kostroma, Russia                      | 58°30'     | 41°40'      | 100                |
| 3   | Vitebsk Polotsk, Belorussia           | 55°30'     | 28°46'      | 200                |
| 4   | Burträsk, Sweden                      | 64°30'     | 20°40'      | 240                |
| 5   | Emmaboda, Sweden                      | 56°38'     | 15°34'      | 160                |
| 6   | Westerhof, Germany                    | 51°48'     | 10°12'      | 200                |
| 7   | Seed orchard 240 Hofmansgade, Denmark | 55°32'     | 10°29'      | 0-10               |
| 8   | Oslofjorden, Norway                   | 58°30'     | 8°55'       |                    |
| 9   | Seed orchard 634 Tvillum, Denmark     | 56°16'     | 9°44'       | 50-100             |
| 10  | Malå, Sweden                          | 65°30'     | 18°45'      | 400                |
| 11  | Sitka spruce, seed orchard 113 Åkulla | 57°08'     | 13°35'      | 90                 |

Table 2. – Chilling treatments in the nursery experiment: date of transfer from outdoors to indoors, chill days (CD) and chilling units (CU) obtained at the date of transfer, average survival, days and temperature sum to reach budburst, and leader length after budburst in treatments T5 to T10. Average over all seed sources.

| Treatment | Date        | CD  | CU   | Days to budburst | TS to budburst | Survival <sup>1)</sup> | Leader length <sup>2)</sup> |
|-----------|-------------|-----|------|------------------|----------------|------------------------|-----------------------------|
| T1        | 29 Aug 2000 | 0   | 0    | 254              | 3929           | 72,4                   | -                           |
| T2        | 12 Sep      | 0   | 1,1  | 241              | 3676           | 80,7                   | -                           |
| T3        | 25 Sep      | 0   | 7,2  | 218              | 3327           | 71,8                   | -                           |
| T4        | 10 Oct      | 1   | 9,0  | 169              | 2585           | 82,1                   | -                           |
| T5        | 23 Oct      | 1   | 11,3 | 87               | 1347           | 91,3                   | 34,0                        |
| T6        | 6 Nov       | 7   | 20,9 | 41               | 638            | 98,1                   | 27,5                        |
| T7        | 21 Nov      | 15  | 32,9 | 34               | 524            | 97,1                   | 33,2                        |
| T8        | 5 Dec       | 23  | 44,3 | 31               | 487            | 96,6                   | 40,8                        |
| T9        | 18 Dec      | 30  | 53,0 | 36               | 548            | 96,7                   | 41,1                        |
| T10       | 17 Apr 2001 | 149 | 89,4 | 16               | 281            | 87,4                   | 59,2                        |

<sup>1)</sup> Percentage of seedlings that survived and reached budburst

<sup>2)</sup> Length of the new leader, mm

The experiment was designed to allow ten different chilling treatments, i.e. ten occasions when seedlings were transferred from outdoor to indoor conditions (Table 2). The seedlings were distributed over six blocks, each with one container per treatment. Each container had 40 seedlings and each of the 11 seed sources were represented by three or four randomly distributed seedlings.

Growth conditions were kept constant in a heated greenhouse with +20°C day and night temperatures and natural light supplemented by artificial light to give 12 h days. Fertilizer was applied when the first seedlings in a treatment started to grow.

The temperature during both the outdoor and indoor phases of the experiment was recorded every 15 to 30 minutes with Tiny tag temperature loggers.

#### Phytotron experiment

The eight seed sources were represented by 24 seedlings per seed source in each of 10 chilling treatments, thus comprising, in all, 1920 seedlings. The seed was sown on 3 February 2000 in plastic tubes (diameter 28 mm, height 150 mm) filled with mineral wool and placed in 24-seedling racks. Three randomly distributed seedlings represented each seed source in each rack. There were five racks per truck and in total 80 racks placed on 16 trucks. Each chilling treatment was applied to eight racks randomized over the 16 trucks in such a way that each chilling treatment set included racks sampled from different trucks and from different positions within the trucks. Thus, an incomplete block design was applied because a single truck could only accommodate five racks, i.e. five chilling treatments. It also means that the design was nearly balanced, in the sense that the occurrence of pairs of chilling treatments within a truck was as equal as possible. A full balance would have required 18 trucks, but the climate chamber could only take 16 trucks.

Seedlings were raised under continuous light from metal halogen Osram HQI-E 250W lamps at 300  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for 10 weeks (Fig. 2). They were then exposed to night treatments, starting with 1 h nights that were successively extended by 1 h week<sup>-1</sup> until they were 12 h long. Thereafter, the night length was kept at 16 h for the rest of the first growth period. The chilling treatment started after seven weeks of 16 h nights, when the temperature was lowered to +5°C for one week, and then to +3°C, which was maintained for the rest of the chilling treatment. The light intensity was lowered to 90  $\mu\text{mol m}^{-2} \text{s}^{-1}$  during the chilling conditions. Nutrients were supplied, from four weeks after sowing, in a complete nutrient solution of low concentration, 100 mg N litre<sup>-1</sup>, based on INGESTAD (1979). The frequency of nutrient supply was dependent on the growth rate.

The chilling requirements for completing the rest phase were tested by removing seedlings from the chilling conditions every week or second week on 10 occasions (giving 10 chilling treatments; Fig. 2). The seedlings were then transferred to another climate chamber with conditions designed to promote growth resumption (a constant temperature of +20°C and continuous light). Before the start of the chilling treatments, the number of seedlings per rack was reduced from 24 to 12 to avoid competition.

#### Data collection

In the nursery, height was measured at the end of the first growing season, and after all the seedlings had set bud. In the phytotron, the height of the epicotyl of a subsample of the seedlings was measured weekly during the first growth period.



ment increased. The seedlings that were left outdoors until April needed only 16 days to reach this stage (Table 2). The main drop in temperature sum requirements occurred around T4 to T6, i.e. at the end of October and early November.

The average height after the first growing season was 126 mm, but there was considerable variation among the seed sources. Burträsk and Malå were the shortest provenances (Table 3). The lowest survival was found for Oslofjorden and Malå, while the others had close to or over 90% survival. Tvilum and Malå had lower leader lengths. The relative importance of the interaction effect between treatment and seed source was small compared to the main effects, although significant results were obtained for survival ( $p=0.015$ ) and leader length ( $p<0.0001$ ).

The temperature sum required for each seed source in relation to previous chilling is shown in Figure 3. In the analysis with model 1, the first two treatments (T1–T2) were omitted. The fixed effects of treatments, seed sources and the interaction between treatment and seed source were significant ( $p<0.0001$ ), but the block effect was not ( $p=0.27$ ). The variance for the random effect main plots was 12462, and the variance of the residuals was 168482. The ratio,  $R^2$ , of the model sum of

Table 3. – Height after the first growing season in the nursery, percentage of the sown seedlings that survived and reached budburst in the greenhouse, and leader length obtained in the 2nd growth period in the greenhouse (average of treatments T6 to T10). Seed sources with the same letter after the height value could not be statistically distinguished at the 5% significance level.

| Seed source    | Height, mm | Survival, % | Leader length, mm |
|----------------|------------|-------------|-------------------|
| 1. Kirov       | 115 e      | 90,7 a      | 41,0 abc          |
| 2. Kostroma    | 128 d      | 91,2 a      | 45,3 ab           |
| 3. Vitebsk     | 148 b      | 89,4 ab     | 48,6 a            |
| 4. Burträsk    | 81 f       | 92,3 a      | 37,8 bc           |
| 5. Emmaboda    | 135 cd     | 92,8 a      | 43,6 ab           |
| 6. Westerhof   | 165 a      | 90,5 ab     | 44,6 ab           |
| 7. Hofmansgade | 146 b      | 93,3 a      | 43,5 ab           |
| 8. Oslofjorden | 118 e      | 82,2 b      | 43,0 ab           |
| 9. Tvilum      | 164 a      | 87,8 ab     | 32,9 c            |
| 10. Malå       | 43 g       | 65,7 c      | 22,0 d            |
| 11. Sitka      | 144 bc     | 86,1 ab     | 37,6 bc           |

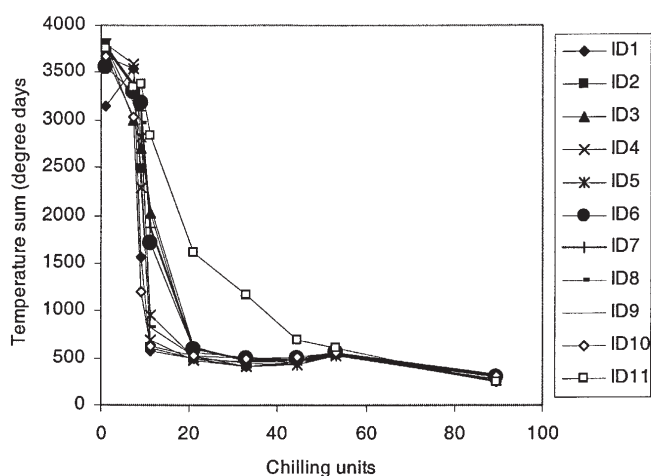


Figure 3. – Temperature sum in the greenhouse required to reach budburst and previous chilling, expressed as chilling units, for each of the 11 seed sources.

squares to the corrected total sum of squares was 0.85, and is used as a measure of the overall determination of model 1.

The results obtained by using the non-linear model (model 3) are summarized in Table 4, and examples of the curve-fitting are shown in Figure 4. The model explained the relationship between TS and CU well ( $R^2=0.80$ ). The  $p$ -values obtained in the tests of equality among the seed sources for the  $a_i$ ,  $d_i$ , and  $c_i$  parameters were 0.97, 0.05, and  $<0.0001$ , respectively. The  $c_i$  parameter for Kirov, Kostroma, Burträsk and Malå (i.e. Russia and northern Sweden) were significantly higher than the corresponding values for Vitebsk, Westerhof, Hofmansgade, Tvilum and Sitka spruce. Emmaboda and Oslofjorden were intermediate in this respect. Chilling demand, expressed as the number of chilling units required for completion of the rest phase (1.05 times the lowest temperature sum), was lowest for the Russian and north-Swedish seed sources ( $CU<=20$ ), highest for the Sitka spruce, and second highest for Vitebsk, Westerhof and the two Danish seed orchards ( $CU>34$ ).

The pair-wise comparisons did not have the same precision, leading in some cases to insignificant results, although the absolute difference was sometimes larger than other differences that were significant.

#### Phytotron experiment

The mortality in the phytotron was negligible. The few seedlings that died were replaced by surplus seedlings, so all

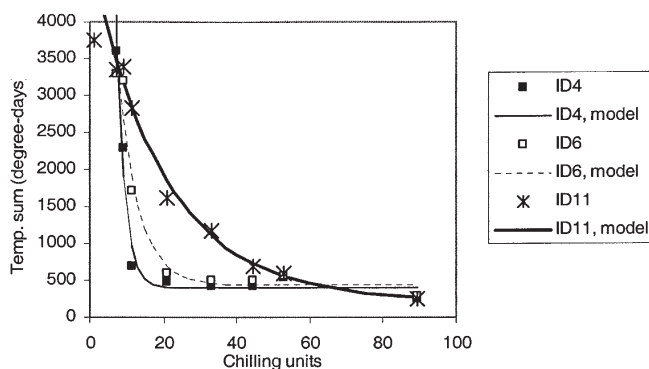


Figure 4. – Examples of curve-fitting for the nursery data. Temperature sums for seed sources 4, 6 and 11 and their curves derived with model 3.

Table 4. – Values of the parameters ( $a_i$ ,  $d_i$ ,  $c_i$ ) in model 3 obtained for treatments T3 to T10 for each seed source, and chilling units required for rest completion (1.05 times the lowest temperature sum of the function). Seed sources with the same letters after these values showed no significant differences in the corresponding parameter, at the experiment-wise error rate of 0.05. The last line gives the P-value for the test of equal parameter values over the seed sources.

| Seed source    | $a_i$ | $d_i$ | $c_i$     | Chilling units, 1.05 |
|----------------|-------|-------|-----------|----------------------|
| 1. Kirov       | 424   | 3173  | 0.584 a   | 15.6 ab              |
| 2. Kostroma    | 413   | 3186  | 0.394 ab  | 19.8 bc              |
| 3. Vitebsk     | 407   | 2855  | 0.151 c   | 39.7 d               |
| 4. Burträsk    | 400   | 3311  | 0.436 ab  | 18.7 abc             |
| 5. Emmaboda    | 415   | 3356  | 0.310 b   | 23.4 cd              |
| 6. Westerhof   | 436   | 3102  | 0.180 c   | 34.5 d               |
| 7. Hofmansgade | 418   | 3134  | 0.167 c   | 37.0 d               |
| 8. Oslofjorden | 399   | 3346  | 0.304 b   | 23.9 cd              |
| 9. Tvilum      | 420   | 2926  | 0.149 c   | 40.2 d               |
| 10. Malå       | 478   | 2493  | 0.709 ab  | 13.6 a               |
| 11. Sitka      | 222   | 3233  | 0.050 d   | 120.7 cd             |
| Equality       | 0.97  | 0.05  | $<0.0001$ | $<0.0001$            |

Table 5. – Chilling treatments in the phytotron experiment: week of transfer of seedlings from chilling to growth conditions, number of chill days (CD) and chilling units (CU), average days and temperature sum (TS) to reach budburst, and leader growth after budburst in treatments T6 to T10. Average over all seed sources.

| Treatment | Week of transfer | CD | CU   | Days to reach budburst | TS to budburst | Leader growth, mm/day |
|-----------|------------------|----|------|------------------------|----------------|-----------------------|
| T1        | 28               | 0  | 0    | 20.6                   | 309            | -                     |
| T2        | 30               | 0  | 0    | 17.9                   | 268            | -                     |
| T3        | 31               | 7  | 5.8  | 17.4                   | 261            | -                     |
| T4        | 32               | 14 | 12.7 | 17.2                   | 257            | -                     |
| T5        | 34               | 28 | 26.4 | 15.5                   | 232            | -                     |
| T6        | 36               | 42 | 40.2 | 15.0                   | 225            | 2.5                   |
| T7        | 38               | 56 | 53.9 | 14.4                   | 216            | 2.5                   |
| T8        | 40               | 70 | 67.7 | 13.6                   | 204            | 2.5                   |
| T9        | 42               | 84 | 81.5 | 13.4                   | 201            | 2.6                   |
| T10       | 43               | 91 | 88.4 | 12.8                   | 192            | 2.5                   |

Table 6. – Height after first growing season (mm) in the phytotron, final shoot length after budburst (mm) and day of budset (days after sowing) for each of the seed sources. Seed sources with the same letter after these values could not be statistically distinguished at the 5% level with Tukey's test.

| Seed source    | Height | Leader length | Day of budset |
|----------------|--------|---------------|---------------|
| 1. Kirov       | 122 e  | 93,6 bc       | 131,0 e       |
| 2. Kostroma    | 134 d  | 95,7 ab       | 134,7 d       |
| 3. Vitebsk     | 149 c  | 87,7 bc       | 139,2 b       |
| 4. Burträsk    | 106 f  | 95,2 abc      | 125,8 f       |
| 5. Emmaboda    | 141 cd | 95,4 ab       | 136,5 c       |
| 6. Westerhof   | 180 a  | 84,8 c        | 146,8 a       |
| 7. Hofmanggade | 163 b  | 93,3 bc       | 145,1 a       |
| 8. Oslofjorden | 132 de | 104,5 a       | 132,7 e       |

pots contained one living seedling at the beginning of the chilling period.

The time and temperature sum required to reach budburst decreased as the length of the chilling treatment increased. However, the effects of chilling were not as pronounced as in the nursery. Temperature sum decreased from 309 degree-days in T1 to 192 degree-days in T10. The growth rate (leader length per day) in the 2<sup>nd</sup> growth period was the same in all treatments. No budset occurred since the seedlings were grown in continuous light.

Figures derived using model (2) for the time of budset, height after the first growth period and leader length when shoot elongation was complete are shown in Table 6. The tallest seedlings were from Westerhof, followed by Hofmanggade, and the shortest from Burträsk. The average day of budset was day 136 (19 weeks after sowing), with individual seedlings varying between day 112 and 165. This implies that all seedlings had set buds well in advance of the start of the chilling treatment.

The temperature sum for each seed source in relation to previous chilling is shown in Figure 5. The fixed effects of treatment, seed source and their interaction were significant ( $p < 0.0001$ ) according to the analysis with model 2. The effects of block ( $p = 0.33$ ), position of rack ( $p = 0.68$ ) and the interactions between both position and treatment ( $p = 0.72$ ) and position and seed source ( $p = 0.17$ ) were not significant. The variance for the random effect main plots was 60.39, and the random residual variance was 1669.2. The  $R^2$  ratio was 0.52.

Excluding the first treatment, the main effects of treatment and seed source were significant ( $p < 0.0001$ ), but their interaction was not ( $p = 0.14$ ). The  $p$ -values for the effects of block (0.80), position of rack (0.66), interaction between position and treatment (0.87), and interaction between position and seed

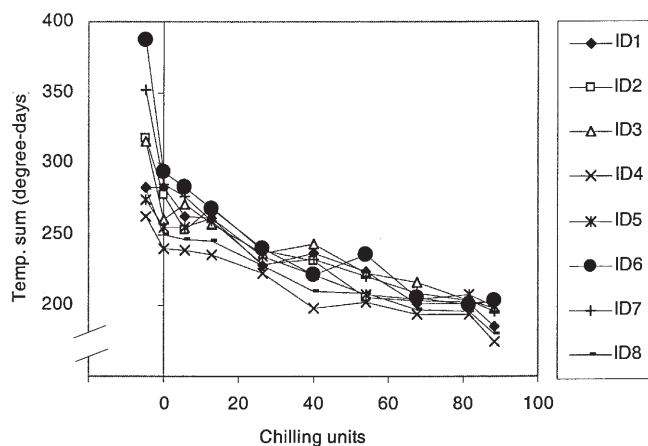


Figure 5. – Temperature sum required to reach budburst and previous chilling in the phytotron for each of the eight seed sources. The data at the left of the 0-axis relate to plants given the T1 treatment, which were moved to growing conditions two weeks before chilling started.

source (0.07) were almost the same as with the first treatment included. The determination coefficient was changed to  $R^2 = 0.44$  since the excluded treatment, one of those with  $CU = 0$ , made a large contribution to the model-explained variation, cf. Figure 5.

Results obtained from the non-linear model (3) for the phytotron experiment are shown in Table 7, and examples of the curve-fitting are presented in Figure 6. The exponential curve was very poorly fitted to the phytotron data ( $R^2 = 0.37$ ). There was no significant effect of seed source on any of the parameters  $a_i$ ,  $d_i$ , or  $c_i$ . Consequently, the calculated chilling requirements for the different seed sources could not be statistically separated, so they are not shown. The lowest temperature sum requirement was shown by Burträsk in all treatments, and the highest in most of the treatments (T1-3, T5, T7 and T10) by Westerhof.

## Discussion

The results from the nursery study corroborated previous studies in which inverse, curved relationships between temperature sum and chilling were found (for a review, see CANNELL and SMITH, 1983). As expected, Sitka spruce had a very high chilling requirement. CANNELL and SMITH (1983) found that Sitka spruce needed over 140 days of chilling to complete its rest phase. The Norway spruce provenances in our study had completed their rest by November-December, in accordance with previous studies indicating that the chilling requirements

Table 7. – Values of the parameters ( $a_i$ ,  $d_i$ ,  $c_i$ ) in model 3 obtained for treatments T2 to T10 in the different seed sources for the phytotron experiment. The last line gives the P-value for the test of equal parameter values over the seed sources.

| Seed source    | $a_i$ | $d_i$ | $c_i$  |
|----------------|-------|-------|--------|
| 1. Kirov       | 177   | 103   | 0.0187 |
| 2. Kostroma    | 175   | 100   | 0.0165 |
| 3. Vitebsk     | 172   | 97    | 0.0120 |
| 4. Burträsk    | 162   | 85    | 0.0156 |
| 5. Emmaboda    | 168   | 92    | 0.0124 |
| 6. Westerhof   | 185   | 110   | 0.0222 |
| 7. Hofmanggade | 180   | 105   | 0.0195 |
| 8. Oslofjorden | 165   | 89    | 0.0145 |
| Equality       | 1.00  | 1.00  | 0.99   |

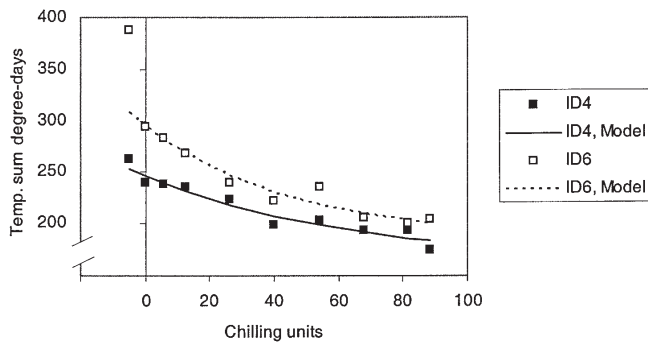


Figure 6. – Examples of curve-fitting for the phytotron data. Temperature sums for seed sources 4 and 6 and their curves derived with model 3.

of Norway spruce are low (NIENSTAEDT, 1967; WORRALL and MERGEN, 1967; HÄNNINEN and PELTONEN, 1988).

The phytotron results did not follow the expected pattern. Even plants given the first treatment, with no chilling, required a relatively moderate temperature sum. The temperature sum decreased with increasing CU, but never leveled out, although the chilling given was of the same magnitude as in the nursery. There are several possible explanations for these results. The seedlings had been growing for several weeks in short-day conditions (16 h night) and QAMARUDDIN et al. (1993) have demonstrated that rest can be broken by long nights, even without chilling. TS decreased considerably between T1 and T2, despite the absence of chilling. Given the relationship shown in Figure 5, it is possible that the data show only part of the critical region of the relationship between chilling and temperature sum, i.e. even the first treatments were sufficient to initiate breakdown of the rest phase. This appeared to be especially true for the northern seed source, Burträsk, and less valid for the southernmost seed source, Westerhof. The results indicate that model 3 is of limited applicability when release from dormancy is well under way. In *Picea abies*, release from dormancy occurred more quickly in long days than in short days after the same amount of chilling in a study by WORRALL and MERGEN (1967). Therefore, inducing a more extended release from dormancy might have enabled us to show variations in chilling requirements amongst seed sources for plants in the phytotron also. The growth conditions, with a constant high temperature of +20°C and continuous light may also have reduced differences amongst the seed sources. HÄNNINEN (1990) showed that the effect of chilling was more pronounced when time to budburst was tested at 12–17°C than at higher (22°C) temperatures.

The results from the nursery support the hypothesis that the more western provenances (i.e. those from Germany and Denmark) have higher chilling requirements, which could be advantageous when trees are growing in more maritime conditions with relatively warm winters. The Russian and north Swedish provenances had lower chilling requirements, as expected. The high chilling requirement of the Belorussian provenance was, however, unexpected. The differences between the western and the northern and Russian provenances were relatively minor: equivalent to some 20 chilling units, corresponding to circa three weeks at +4°C. Even if there are variations amongst provenance, the data show that Norway spruce is a species with generally low chilling requirements. LEINONEN (1996) found much larger differences between inland (Finland) and coastal (Scotland) provenances of Scots pine and silver birch. The differences in LEINONEN's study were largest under natural chilling conditions, in which the Scots pines from Scot-

land needed 100 days of chilling and those from Finland only 40. The chilling requirements in Norway spruce are probably fully satisfied before the end of December in normal winter conditions in southern Scandinavia. Therefore, factors other than chilling requirement might explain provenance differences in vulnerability to winter damage.

Frost desiccation, or winter damage, describes the type of damage observed in early spring, when needles turn red after periods of cold nights and clear days with increasing needle temperatures. The reasons for the damage vary: it may result from frost injury that occurred in the previous autumn, from desiccation or from freezing injury in tissues that have dehardened after a warm spell. Several observations indicate that varieties with late growth cessation suffer most from frost desiccation. For instance, HANNERZ (1994) found that Belorussian provenances were more prone to injury by frost desiccation than Swedish provenances. Similarly, SKRØPPA and DIETRICHSON (1986) observed more winter damage amongst western continental spruce provenances than amongst Scandinavian provenances. Also, DANUSEVICIUS and GABRIVALICIUS (2001) found that provenances with late flushing and late growth-cessation were less tolerant to frost desiccation. Corresponding results have been shown in *Picea mariana* [Mill.] B.S.P. (STÄHL and PERSSON, 1992), *Picea pungens* Englm. (BONGARTEN and HANOVER, 1986) and *Pinus sylvestris* (VAN HAVERBEKE, 1979). These observations conflict with the idea that seed sources with a high chilling requirement should be more tolerant of winter damage. Instead, they suggest that varieties should be selected that cease growth early and are likely to reach a high hardiness level during the autumn. The risk of dehardening during warm periods in the winter, and its relationship with the state of rest, remains to be explored in Norway spruce.

Warmer winters as a result of climate warming are likely to affect the timing of the onset and cessation of growth in many ways (KRAMER, 1995). The risk of damage depends on complex interactions between the hardening response in the autumn and the requirements for chilling and temperature sum to induce budburst. CANNELL and SMITH (1986) expected that warmer winters would increase the risk of insufficient chilling, thereby delaying budburst, while warmer spring temperatures would induce earlier budburst. MURRAY et al. (1989) predicted in a British study that tree species with a low chilling requirement would flush earlier at a high altitude site, but not at a low altitude site they examined. HÄNNINEN (1995) stressed that models developed to predict effects of climate change must be realistic. Models that have not been tested under natural conditions may give erroneous predictions in situations where they insufficiently reflect environmental relationships. However, the role of fluctuating hardiness and risk of damage before budburst is poorly understood, so these models require considerable further refinement.

Neither the empirical study nor the statistical model was designed to compare the temperature sum required at full chilling. The  $a$ -parameters were not significantly different in the two experiments. However, provenances differed significantly in TS in many individual treatments. The ranking was relatively stable in the phytotron, with the northern Burträsk having the lowest temperature sum requirement and Westerhof the highest. The ranking mirrors the climatic adaptation of the provenances. A high temperature sum requirement prevents budburst occurring too early in trees growing in a maritime climate with warm winters and extended spring periods. In a more northern and continental climate, temperature rises quickly in the spring and there is less risk of temperature falling back to damaging levels after budburst. In such condi-

tions it is advantageous to start growth early to take advantage of the shorter growing season. Hence, the temperature sum requirement is lower.

In conclusion, the chilling requirements of the Norway spruce seedlings had generally been fulfilled by November under natural chilling conditions. Although differences amongst provenance were observed, they were relatively minor. Also, the observed differences supported the hypothesis that western provenances had high chilling requirements while northern and eastern provenances lower chilling requirements. An exception was the Belorussian provenance, which had a high chilling requirement. Phytotron studies were not able to detect differences in chilling requirements, since the rest began to break even before chilling was initiated. This demonstrates that rest break is a complex process, governed by several factors that need to be thoroughly elucidated in order to develop realistic models of the effects of potential changes such as climatic warming.

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