

Climatic adaptation of bud set and frost hardiness in Scots pine (*Pinus sylvestris*)

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Abstract: The variation of timing of terminal bud set and frost hardening in first-year seedlings of Scots pine (*Pinus sylvestris* L.) was studied. Both bud set and frost hardiness showed clinal variation with respect to latitude over a steep environmental gradient (60–67°N) in Finland. Northern populations set buds and developed frost hardiness earlier than southern populations. Backcrosses ((north × south) × south) were intermediate or close to the southern parent both in bud set and in frost hardiness between the populations representing the parental origins. In bud set, 36.4% of the total variation was between the populations. The bud set date and development of frost hardiness were highly correlated at the population level ($r = 0.69–0.97$). Frost hardening started during the bud formation period and accelerated when most of the buds had formed. In backcross progenies, earlier bud set resulted also in earlier development of frost hardiness. This suggests a genetical association between the two traits.

Résumé : La variation dans le moment où le bourgeon terminal est formé et la résistance au gel est acquise a été étudiée chez des semis de pin sylvestre (*Pinus sylvestris* L.) âgés de 1 an. La formation du bourgeon terminal et la résistance au gel montraient une variation clinale par rapport à la latitude le long d'un fort gradient environnemental (60 à 67°N) en Finlande. Les populations septentrionales formaient leur bourgeon terminal et devenaient résistantes au gel plus tôt que les populations méridionales. Les populations issues de rétrocroisements ((nord × sud) × sud) se situaient à mi-chemin ou près des parents d'origine méridionale pour la formation du bourgeon terminal et la résistance au gel comparativement aux caractéristiques des populations dont provenaient les parents. Dans le cas de la formation du bourgeon terminal, la variation entre les populations représentait 36,4% de la variation totale. La date à laquelle le bourgeon terminal était formé et l'acquisition de la résistance au gel étaient fortement corrélées ($r = 0,69–0,97$) au sein des populations. L'acquisition de la résistance au gel débutait pendant la période de formation du bourgeon terminal et s'accélérait lorsque la plupart des bourgeons étaient formés. Dans les descendance issues de rétrocroisements, l'acquisition de la résistance au gel survenait plus tôt si le bourgeon terminal se formait plus tôt. Les résultats suggèrent qu'il existe une association d'ordre génétique entre les deux caractères.
[Traduit par la Rédaction]

Introduction

Scots pine (*Pinus sylvestris* L.) has the widest range of all pine species (Mirov 1967), extending in Europe from Spain to northern Scandinavia. In Finland alone, the length of the thermal growing season (days with average temperature above 5°C) varies from about 170 days at latitude 60°N to less than 120 days at latitude 69°N. Growing in diverse environmental conditions is possible through plastic responses or genetic differentiation. Indications of local genetic adaptation are obtained from transfer experiments. When southern provenances are transferred to the north, their survival is reduced (Eriksson et al. 1980; Persson 1994; Beuker 1994). Northern provenances transferred to the south have increased survival and growth, but their growth is slower than that of local southern provenances (Beuker 1994).

Trees time their growth and reproduction to coincide with favorable conditions. In late summer or in the fall, vegetative growth ceases, terminal buds are formed, and frost hardiness begins to develop. Generally, the cessation of growth takes

place before the onset of low temperatures. The exact roles of accumulation of temperature sum and decreasing daylength in governing bud formation are still debated (Ekberg et al. 1979; Eriksson et al. 1978; Koski and Sievänen 1985; Hänninen et al. 1990). Northern populations set buds earlier than the southern ones both under natural conditions and when grown in the same environment (common garden experiments), indicating genetic differences in the timing of bud set between populations (Mikola 1982; Karhu et al. 1996). The same is true for the development of frost hardiness, which takes place earlier in northern than in southern populations (Toivonen et al. 1991; Aho 1994; E. Beuker, E. Valtonen, and T. Repo, unpublished). Hardening starts after bud formation (Weiser 1970; Colombo et al. 1989) and is influenced both by shortening daylength and by exposure to low temperatures (Christersson 1978).

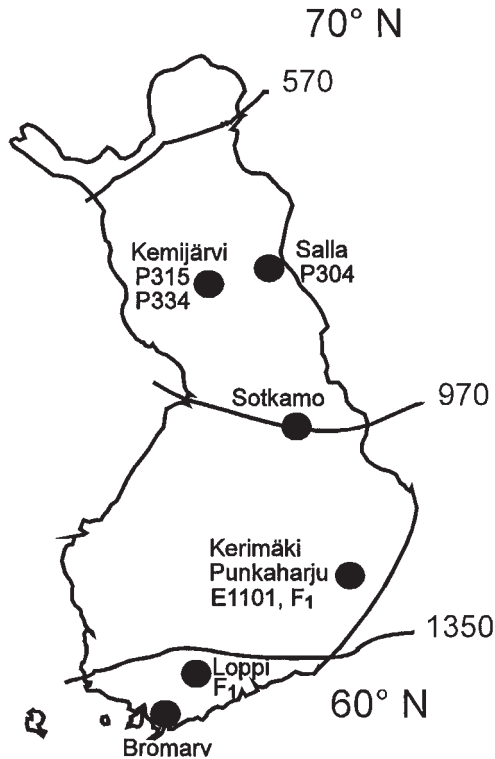
Earlier studies have thus shown that there are clines in the timing of terminal bud set and in the development of frost tolerance, both of which represent important adaptations to prevailing climatic conditions. Such parallel clines could be due to several factors (Endler 1977). First, they could be a consequence of natural selection on both traits independently. Second, correlated patterns of variation could also be due to a partly shared genetic basis, due to either linkage or pleiotropy. Here, our purpose is to examine the relationship of these two clines in two ways. First, we study the same populations simultaneously for both traits. Second, by studying the patterns of variation in progeny of crosses, we gain further knowledge

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Fig. 1. Origins of Finnish populations of Scots pine (Salla, Sotkamo, Kerimäki, and Bromarv), parents of the backcrosses (P304, P315, P334, E1101), and locations of the F₁ trees. F₁ Punkaharju = P304 × E1101 in 1993; F₁ Loppi = P334 × E1101 and P315 × E1101 in 1994. Average temperature sums of growing seasons are also depicted.



of the association versus independence of these traits. These studies will form the basis for later, more detailed genetic studies.

Material and methods

Population samples

Four population samples of Scots pine seedlings from different latitudes in Finland (Salla, Sotkamo, Kerimäki, and Bromarv) were included in the study (Fig. 1.). Seeds for the experiments were bulked samples from the forests of the Finnish Forest Research Institute.

Crossing material

Two backcross progenies were included in the years 1993 and 1994. Crosses were between the trees from different parts of the environmental gradient in Finland. The material of 1993 was from a cross between a northern plus tree P304 from Salla (maternal tree, P represents north) and a southern plus tree E1101 from Punkaharju (paternal tree, E represents south). Progeny F₁ trees from the cross grow in Punkaharju, southern Finland (Fig. 1). Open pollinated seeds from two F₁ trees were collected. These are regarded as backcrosses to southern pollen surrounding the F₁ trees.

In 1994 the experiment consisted of two backcross progenies originating from different maternal trees of northern Finland. The first cross was P334 (Kemijärvi, maternal tree) × E1101 (Punkaharju, paternal tree) and the other was P315 (Kemijärvi,

maternal tree) × E1101. Progeny F₁ trees from these crosses grow in Loppi, southern Finland (Haapastensyrjä Tree Breeding Centre) (Fig. 1). Open pollinated seeds from the two F₁ trees were collected, assuming again that they are similar to backcrosses to southern trees. Throughout this paper, (P334 × E1101) × E is designated as backcross 1 and (P315 × E1101) × E as backcross 2. Note that in 1993, material was from the same cross; here, it is from two different crosses.

Seedling production

The seedlings for the experiments were raised in a greenhouse at the Finnish Forest Research Institute, Punkaharju Forest Research Station (61°48'N, 29°19'E; 90 m above sea level) in the years 1993 and 1994. From each population and from each F₁ tree, 450 seeds were sown in prefertilized peat mould (Vapo) in 10 trays of 45 cells on 1 June 1993 and 1994. The size of the cell was 5.0 × 5.4 × 10 cm (depth). The trays were set in 10 blocks, each block containing one tray of each population and backcross progenies. Seedlings were grown in a greenhouse under natural light conditions and temperature following ambient temperature, except that temperature was not allowed to fall below 5°C. In 1994, from June until the middle of August the temperature in the greenhouse ranged from 17 to 27°C during the day and from 13 to 20°C during the night. From the middle of August to the end of September the respective temperatures were 10–22 and 6–14°C and in October were 6–12 and 5–9°C.

Bud set and growth

The timing of terminal bud set was scored twice a week from the beginning of August until the end of October. The bud was regarded as formed when it was seen clearly between the needles above the seedling. The timing of bud set was given as the number of days from sowing to the date of bud set. Differences in the timing of terminal bud set between the populations were analysed with a two-way analysis of variance (ANOVA) (Sokal and Rohlf 1981), regarding populations and blocks as random effects. The proportion of variation between populations and blocks was also estimated. The analyses were done with the SAS/STAT computer software (SAS Institute Inc. 1987).

The height of the seedlings was measured twice to monitor growth. The first measurement was done on 17 August and the second on 10 October at the end of the growing season to get the final shoot length. The proportion of total growth reached at the time of the first measurement was calculated for 1994.

Frost hardiness

Frost hardiness was assessed starting on 29 August 1994 and continued until 7 October 1994 at 2-week intervals. Five to six seedlings were selected at random from each tray at each time. A total of 56 seedlings for each population and backcross progeny were used in each assessment.

Frost hardiness was based on controlled freezing treatments in air-cooled chambers. On each occasion, six different frost temperatures (eight seedlings per treatment temperature) were used, and the control was always 5°C (Table 1). The freezing temperatures were chosen to induce different degrees of damage. The freezing exposure program started at 10°C and was gradually cooled to the target temperature at a rate of 5°C/h. The minimum temperature phase lasted 4 h, after which the temperature was raised back to 10°C gradually at 5°C/h. The

Table 1. Treatment temperatures used for frost hardiness assessment on seedlings of Scots pine populations and backcross progenies on each date in 1994.

Date	Treatment temperatures (°C)						
29 Aug. – 1 Sept.	5	-2	-4	-6	-8	-10	-12
12–14 Sept.	5	-3	-6	-9	-12	-15	-20
26–28 Sept.	5	-6	-10	-14	-18	-20	-26
10–12 Oct.	5	-6	-10	-16	-22	-28	-34
24–26 Oct.	5	-8	-16	-24	-32	-40	-48
7–9 Nov.	5	-8	-16	-24	-32	-40	-48

damage to the seedlings after freezing treatment was assessed by three methods: (1) the electrolyte leakage method on needles, (2) visual scoring on needles, and (3) impedance analysis as extracellular resistance on stems.

Frost hardiness assessment by the electrolyte leakage method on needles was carried out as described previously (Flint et al. 1967; Burr et al. 1990; Repo et al. 1994). Briefly, 10-mm samples were cut from the middle of the needles. The samples were put in the tubes (16 samples per tube, four tubes per temperature) with 0.5 mL of distilled water. Frost treatment in air-cooled chambers was conducted as described above. After treatment, 5.5 mL of distilled water was added into the tubes which were set in a shaker (100 rpm) for 22 h before measurement of conductivity. The cells were killed by heating the tubes at 90°C for 15 min before the next 24-h shaking period and before the second measurement of conductivity. The index of injury (I) was calculated as

$$[1] \quad I = \frac{T_1 C_2 - T_2 C_1}{T_2 (C_2 - C_1)}$$

where T_1 and T_2 are conductivities of the water solution after the first and second shaking period, respectively, and C_1 and C_2 are the mean conductivities of the control samples (5°C) after the first and second shaking period, respectively. Frost hardiness was estimated as the inflection point (parameter C) of eq. 2 fitted to the temperature (x) response data of index of injury (y):

$$[2] \quad y = \frac{A}{1 + e^{B(C-x)}} + D$$

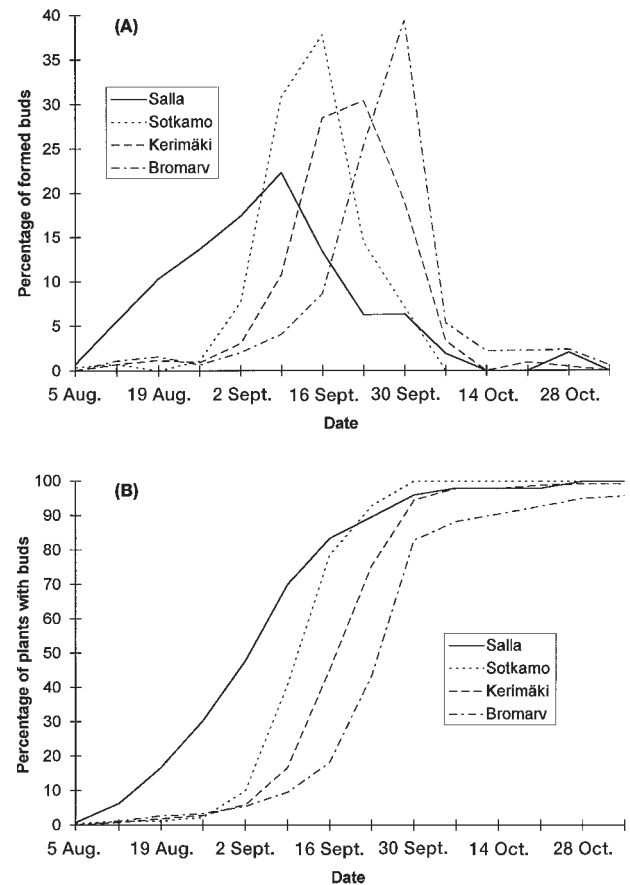
where A , B , C , and D are constants.

For visual damage scoring, after treatment, a piece of about 4 cm was cut from the top of all frost-treated and control seedlings. The sample was set in tap water in a cup and transferred to the greenhouse (20°C, 16 h day : 8 h night). After 2 weeks, visual damage was scored in five classes, each with a 20% interval, based on the browning of the needles. The temperature at which 50% of the needles survived was obtained by nonlinear regression of Statgraphics (Manugistics, Inc., Rockville, U.S.A.) of the logistic sigmoid function (eq. 3) fitted into the temperature response data of the visually scored damage:

$$[3] \quad y = \frac{1}{1 + e^{b(c-x)}}$$

where y is the visually scored damage, x is the frost treatment temperature, and b and c are constants. The inflection point (parameter c) was taken as the frost hardiness estimate.

Fig. 2. (A) Distributions of terminal bud set and (B) cumulative distribution of terminal bud forming in first-year seedlings of Scots pine populations from different latitudes in Finland in 1994.



Frost hardiness of stems was assessed by impedance analysis as described previously (Repo et al. 1994). After the freezing treatment (eight seedlings per temperature), a 15-mm section was cut from the middle of the stem. The electrical impedance spectrum (80 Hz to 1 MHz) was measured with an HP4284A impedance analyzer. Equivalent circuit parameters of the distributed circuit model (double-DCE) were estimated with the CNLS-program (LEVM v. 6, Scribner Associates, Inc., Charlottesville, U.S.A.). As a measure of frost hardiness, the inflection point (eq. 2, parameter C) was estimated from the temperature response of the extracellular resistance by nonlinear regression of Statgraphics software.

Relationship between bud set and frost hardiness

The relationship between bud set and frost hardiness at the population level was examined based on the curves describing development of frost hardiness as assessed by the electrolyte leakage method and the proportion of seedlings that had not yet formed buds. The association between the traits was tested with regression analysis, in which frost hardiness (date of reaching frost hardiness of -10 or -15°C) was used as a dependent variable and the median date of bud set (date of 50% of bud formation) as an independent variable.

Table 2. Median date of terminal bud set, number of days from sowing to median date of bud set, and phenotypic standard deviation in seedlings of four Scots pine populations and in the progenies of backcrosses 1 ((P334 × E1101) × E) and 2 ((P315 × E1101) × E); average heights on 17 August and 10 October 1994; and proportion of total growth on 17 August 1994.

Populations and crosses	Median date of bud set	Days to median date	Phenotypic SD	Height (mm) in August	Height (mm) in October	Proportion of growth
Bromarv	24 Sept.	115	13.0	65.3	83.4	0.78
Kerimäki	17 Sept.	108	10.4	70.2	88.0	0.80
Sotkamo	10 Sept.	101	8.8	62.6	76.8	0.82
Salla	2 Sept.	93	13.1	51.5	60.3	0.85
Backcross 1	19 Sept.	110	11.8	62.7	78.1	0.80
Backcross 2	12 Sept.	103	10.3	73.6	87.8	0.84

Table 3. ANOVA for timing of terminal bud set between Scots pine populations.

Source of variation	df	Mean square	<i>F</i>	<i>P</i>	Variance component (%)
Population	3	20 728.9	84.40	<0.001	36.4
Block	9	209.7	0.85	>0.05	0.09
Population × block	27	245.6	1.90	<0.01	
Error	980	129.6			

Results

Bud set and growth

The distributions of the dates of bud set in 1994 show that the northernmost population (Salla) set terminal buds about 22 days earlier than the southernmost one (Bromarv) (Fig. 2; Table 2). These differences were statistically significant (Table 3). There was significant interaction between the populations and blocks. Of the total variation, 36.4% was found to be between the populations. All populations had large phenotypic variances of bud set, suggesting that there is also genetic variation within populations. The ranking between the populations was similar in 1993 (data not shown).

In the 1994 data, the median date of bud set in backcross 2 was intermediate between the populations from Salla and Kerimäki, which represent the populations of the origins of the parents of the F_1 tree (Table 2). Backcross 1, however, was later than Kerimäki, the origin of the southern parent, but the difference was not significant (ANOVA, data not shown). These results can be observed also from the cumulative progress of bud set (Fig. 3A). Two backcross progenies from the same family, (P304 × E1101) × E, from 1993 were both intermediate between Salla and Kerimäki in bud set (Fig. 3B). The backcrosses were also very similar to each other in bud set.

The northern populations had reached a slightly higher proportion of their total height at the time of the first measurement on 17 August than the southern ones, indicating that the northern populations ceased their growth earlier than the southern ones (Table 2). Thus, the growth pattern corresponded to the timing of bud set. Shoot elongation ended after bud set (data not shown).

Frost hardiness

All results of frost hardiness are at the population level. As measures were not based on individual seedlings, within-population variance could not be estimated. Since the frost hardiness esti-

mates of needles from the electrolyte leakage method and visual scoring were very similar (correlation coefficient (r) = 0.98), we present only results from the former method.

At the time of first assessment, the frost hardiness estimates of needles (electrolyte leakage method) and stems (impedance analysis) were between -5 and -8.5°C (Figs. 4 and 5) for populations and backcrosses, with small differences between organs. The northernmost population from Salla was the most hardy whereas the populations from Bromarv and Kerimäki and backcross 1 were the least hardy.

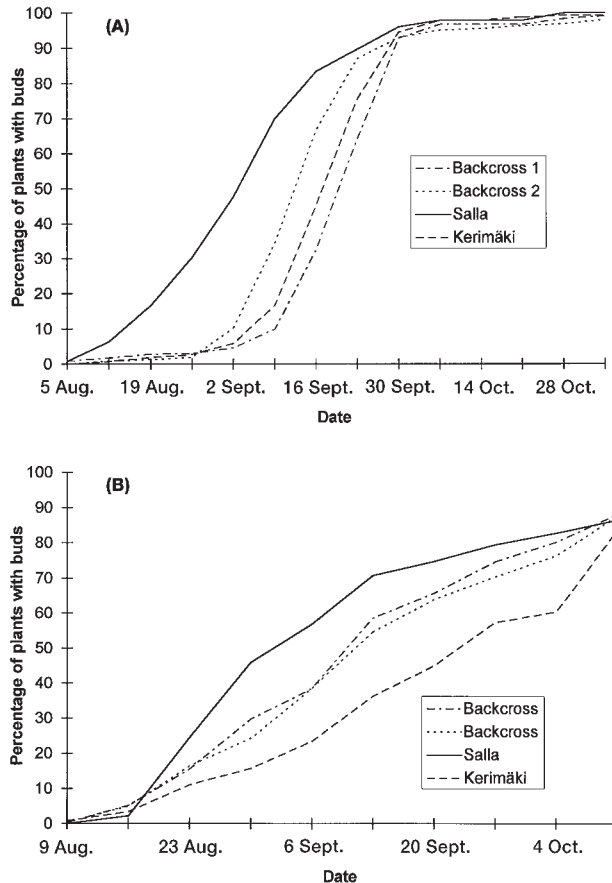
The northernmost population (Salla) started to harden the earliest and the southernmost population (Bromarv) the latest. Once initiated, hardening in needles was faster than in stems (Fig. 4). Hardening in other populations and backcrosses was intermediate between that in Salla and Bromarv (Figs. 4 and 5), and the ranking between populations and backcrosses was approximately similar for both stems and needles. The needles of backcross 2 were a few degrees more frost hardy than those of backcross 1 for most of the hardening period (Fig. 5). The same was found for stems (data not shown). Frost hardening in both backcrosses proceeded in the same way as in the population from Kerimäki.

The frost hardiness of needles increased during the whole period of assessment, and no constant stationary level of hardiness was reached at the end of the experiment (Fig. 4A). The maximum hardiness level of needles in all populations and backcrosses was below -30°C , being about -40°C in the population from Salla. Frost hardiness in stems stabilized at -15°C in all populations except in the northernmost one (about -20°C) (Fig. 4B). The rankings of the populations and backcrosses were similar in stems and in needles. The estimates of frost hardiness differed between the needles and the stems, but there was a linear relationship between them ($r = 0.88$).

Relationship between bud set and frost hardiness

When most of the buds had formed (30 September), frost hardiness developed more rapidly (Fig. 6). Coefficients of determination (R^2)

Fig. 3. (A) Cumulative distribution of terminal bud forming in first-year seedlings of Scots pine in backcrosses 1 ((P334 × E1101) × E) and 2 ((P315 × E1101) × E) and in populations from Salla (located near P334 and P315) and Kerimäki (located near E1101) in 1994. (B) Cumulative distribution of terminal bud forming in first-year seedlings of Scots pine in two different backcross progenies ((P304 × E1101) × E) and in populations from Salla (located near P304) and Kerimäki (located near E1101) in 1993.



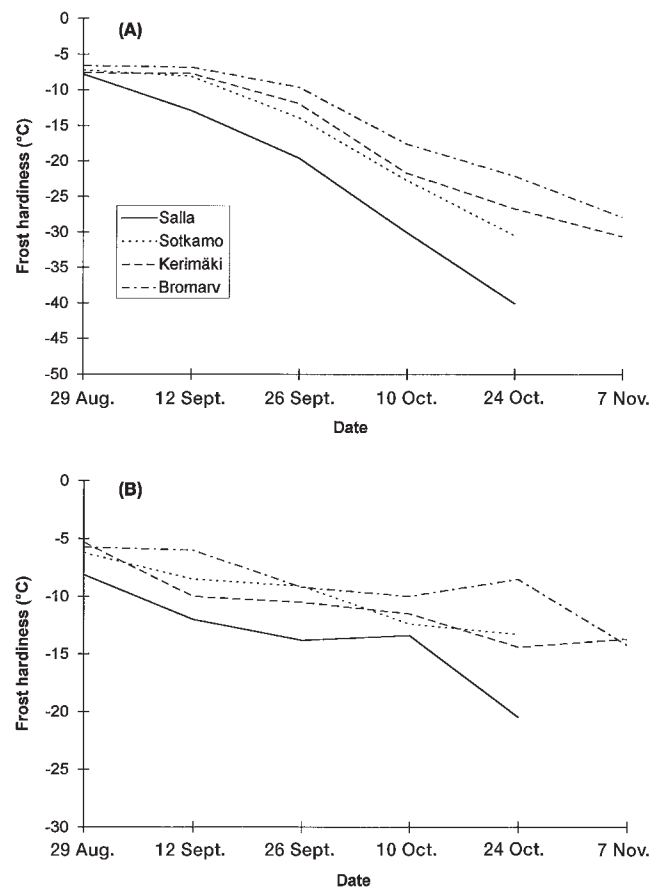
and r between median date of bud set of the populations (date of 50% of bud formation in weeks) and frost hardiness (date of reaching frost hardiness of -10 or -15°C in weeks) were high (Table 4). Correlation in needles was significant, but not in stems (the number of observations was low). These estimates are between population or seed lot correlations, reflections of a shared cline.

Discussion

Latitudinal clines in bud set and frost hardiness

The date of bud set varied latitudinally between the populations of Scots pine first-year seedlings with a difference of about 3 weeks between the northernmost and southernmost population. On average, every increment of 1° of latitude caused bud set about 3 days earlier. Mikola (1982) found twice as steep clinal variation in first-year seedlings of Scots pine from different latitudes in Finland. A different timing of the experiment in relation to the growth period might explain the different results between these studies, as Mikola's study began a month earlier (1 May) than ours. Similar clinal variation

Fig. 4. Development of frost hardiness in (A) needles (electrolyte leakage method) and (B) stems (impedance analysis as extracellular resistance) of first-year seedlings of Scots pine populations.



in bud set and growth has been reported in many other temperate tree species of the Northern Hemisphere as well, e.g., *Picea abies* (L.) Karst. (Heide 1974; Skrøppa 1982), *Picea mariana* (Mill.) BSP (Morgenstern 1978), and many angiosperms. Vaartaja (1959) found photoperiodic ecotypes of growth rhythm in 38 tree species. These findings emphasize the generality of the adaptation.

Our study was on first-year seedlings of Scots pine. However, it would be important to know whether the timing of bud set shows a similar pattern at later stages of the life cycle. During the first growing season the growth of conifers is free and predetermined, but in the following years is mainly predetermined (Jablanczy 1971; Lanner 1976). In *Picea abies*, the bud set dates of populations of 1- to 6-year-old seedlings were correlated over years (Ununger et al. 1988; Ekberg et al. 1994). In adult trees, evidence of similar correlations of growth cessation with latitude has been obtained in *Pinus contorta* Dougl. ex Loud. and *Picea sitchensis* (Bong.) Carrière (Cannell and Willet 1975). Thus, one might expect that the differences between the populations at the seedling stage in Scots pine are also found in older populations. However, natural selection could cause changes in means (directional selection), or stabilizing selection could reduce variances, further emphasizing the differences between populations.

Latitudinal variation in frost hardiness was found between the populations of first-year Scots pine seedlings. Similar findings

Fig. 5. Development of frost hardiness in needles of first-year seedlings of Scots pine by the electrolyte leakage method in backcrosses 1 ((P334 × E1101) × E) and 2 ((P315 × E1101) × E) and in populations from Salla (located near P334 and P315) and Kerimäki (located near E1101).

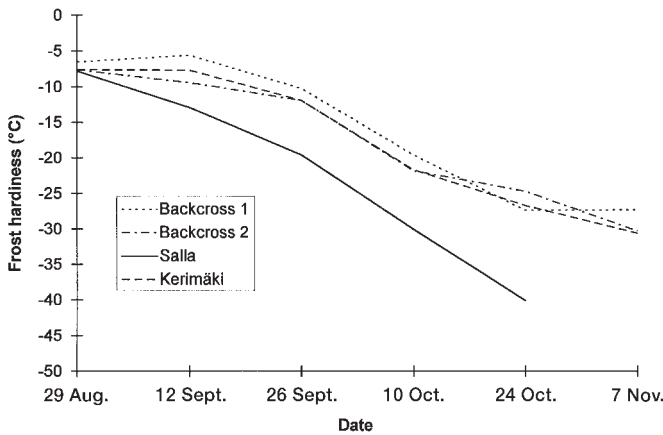
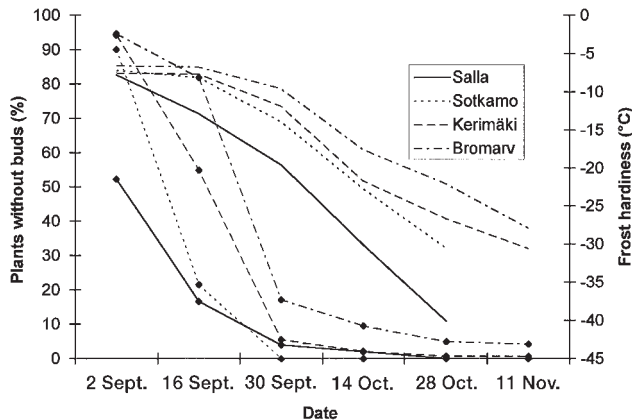


Fig. 6. Relationship between bud setting (percentage of seedlings without buds) (lines with diamonds) and frost hardening of needles (lines without diamonds) of Scots pine seedlings assessed with the electrolyte leakage method.



have been reported previously in Scots pine seedlings (Toivonen et al. 1991; Aho 1994; Hagner 1970) and in *Picea abies* (Ekberg et al. 1979), *Pinus contorta* (Hagner 1970; Jonsson et al. 1981), and *Picea glauca* (Moench) Voss (Simpson 1994), confirming the importance of adaptation.

Clinal variation of frost hardening was steeper in the needles than in the stems. This might indicate that the responses to the changing environmental conditions during autumn differ between needles and stems. In our experiment the photoperiod was naturally declining whereas temperature was not allowed to fall below 5°C. Thus, needles of the first-year seedlings may have responded to decreasing photoperiod with a steady increase of frost hardiness. Stems, on the other hand, may have responded to the lowering temperature, which then prevented further hardening. In a previous study of Scots pine saplings, photoperiod and temperature were both naturally declining, and a similar frost hardening pattern was found in the needles (electrolyte leakage method) and stems (impedance analysis) of current-year shoots (Repo et al. 1995). Different frost hardening pattern of needles and stems in our study should not be

Table 4. Regression analysis of timing of bud set and population frost hardiness of needles and stems.

Organ	R^2 (%)	r	P
Frost hardiness of -10°C			
Needles (electrolyte leakage)	94	0.97	0.00
Stems (impedance analysis)	48	0.69	0.13
Frost hardiness of -15°C			
Needles (electrolyte leakage)	88	0.94	0.01

Note: The dependent variable is the date of frost hardiness of -10°C or the date of frost hardiness of -15°C and the independent variable is the median date of bud set (50% of buds formed). Values of R^2 (coefficient of determination), r (correlation coefficient), and P (probability level) are given.

due to assessment methods either, since both electrolyte leakage and impedance analysis are based on leaching of intracellular ions to the extracellular space.

Variation within populations

Within-population variation for bud set date could also be examined, as these were observed for each seedling individually. Much variation was found in populations and the values were normally distributed. The northern population from Salla had at least equal amounts of phenotypic and presumably genetic variation as the more central populations. Conditions in the greenhouse may have had an impact on the population from Salla, as it grew furthest from its natural growing site. Hence, the different combination of daylength and temperature might have caused genotype environment interaction (Koski and Sievänen 1985). However, high within-population variation in the duration of growth is also typical of other northern forest tree species, e.g., *Picea abies* (Ekberg et al. 1985) and *Picea mariana* (Parker et al. 1994). Moderate and high heritabilities have been observed in different ages in *Pseudotsuga menziesii* (Mirb.) Franco (Li and Adams 1993). Some authors have suggested that marginal populations in harsh environment might lose their variation due to directional selection, as, for example, in *Abies sachalinensis* Mast. (Sakai and Larcher 1987; see also Savolainen and Hurme 1997 for discussion), but this did not seem to be the case here.

Inheritance of bud set and frost hardiness

Backcross progenies allow some conclusions on the inheritance of bud set and frost hardening. We have not characterized the individual parents, just their populations, nor the F_1 distribution, due to the limitations of available material.

In bud set, one backcross was intermediate between the populations of parental origin, Salla and Kerimäki in 1994. The distribution was closer to Kerimäki, which was expected, as the backcross was to southern pollen, but the other backcross was somewhat later in bud set than the population from Kerimäki. Progeny from crosses between provenances generally show intermediate timing of bud set when compared with parents, but dominance has also been found (Eriksson et al. 1978; Junttila 1982; Mikola 1982). Mother trees P315 and P334 were both from Kemijärvi (about the same latitude with Salla), but could differ genetically due to within-population variation. Mother tree P334 might have later genes of bud set compared with P315, the mother tree in backcross 2, causing the strong southward direction in backcross 1. In 1993, two F_1 trees,

originating from the same family (P304 × E1101), had similar progeny bud set dates, suggesting that the southern pollen is genetically homogeneous with respect to bud set dates.

The frost hardening pattern in backcrosses was in agreement with bud set dates, so that backcross 2 was very similar to the population from Kerimäki and backcross 1 later than Kerimäki on most assessment dates. Further implications on these results are discussed below.

Relationship between bud set and frost hardiness

Development of frost hardiness started during the bud formation period, but was accelerated when most of the buds (about 90%) had formed. A similar relationship was also found in *Picea mariana* (Colombo et al. 1989).

High correlations between bud set and initiation of frost hardening found at the population level (0.69–0.97) were similar to findings in, for example, *Picea abies* (Johnsen and Apeland 1988) and in different studies on *Pseudotsuga menziesii* (Campbell and Sorensen 1973; Rehfeldt 1979; Loopstra and Adams 1989). These results indicate that under these conditions (natural photoperiod and temperature conditions), bud set predicts initiation of frost hardening at the population level. This relationship might not be as clearly observable at the family level, as was found, for example, in *Picea abies* (Johnsen and Apeland 1988; Skråppa 1991). However, the traits were correlated at the individual seedling level (Skråppa 1991). Measurements of bud set and frost hardiness on individual seedlings will also be feasible in Scots pine for improved understanding of their relationship.

The parallel clines of bud set and frost hardening found in this study have also been observed previously in *Picea abies* (Ekberg et al. 1979), *Cornus stolonifera* Michx. (Smithberg and Weiser 1968), and *Tsuga heterophylla* (Raf.) Sarg. (Kuser and Ching 1980). Parallelity can be a reflection of selection of both traits independently (e.g., see Endler 1977), so that different genes respond to similar environmental stimuli.

On the other hand, the similar patterns of variation could be due to a shared genetic basis. Some cautious interpretations about the genetical background of bud set and frost hardiness can be made using results of backcross progenies. Polygenic inheritance has been suggested for both traits (Mikola 1982; Eriksson et al. 1978; Norell et al. 1986). Recombination in the F₁ did not lead to completely independent segregation of the two traits, as progeny of backcross 1 were later both in bud set and in frost hardiness when compared with the population from Kerimäki, and backcross 2 was earlier for both traits. This suggests that there is a genetical association between the traits. The genes may be partly the same, or different genes affecting both traits may be linked. Our current crossings and mapping experiments will tell more about the genetical background and the number of the genes influencing the traits.

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