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Frost hardiness development and lignification of young Norway spruce seedlings of southern and northern Finnish origin

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TIIVISTELMÄ: ETELÄ- JA POHJOISSUOMALAISTA ALKUPERÄÄ OLEVIEN KUUSENTAIMIEN PAKKASKESTÄVYYDEN KEHITTYMINEN SEKÄ RUNGON PUUTUMINEN

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Seedlings from four Norway spruce (*Picea abies* (L.) Karst.) stands originating from areas with effective temperature sums ranging from 710 d.d. to 1150 d.d. were raised under artificial light and temperature treatment. After a 10-week growing period the hardening process was started by subjecting the seedlings to +8 °C night temperature and +15 °C day temperature, and increasing the night length by 1.5 hour/week. Hardiness was measured by means of artificial freezing treatment (–10 °C or –15 °C), followed by visual estimation of the degree of needle injury. The stem height, lignification and bud development were measured before the freezing treatment. The amount of injury increased with increasing southernness of the origin of the tested material. Furthermore, the proportion of non-lignified part of the seedling stem was negatively correlated with the latitude of the provenances. The proportion of seedlings with clearly visible buds was more than 90 % in the northernmost entry and less than 1 % in the southernmost one. The overall correlation coefficient between the needle injuries and the proportion of non-lignified part of the stem was rather high, but varied considerably from 0.3 in the northernmost material to over 0.6 in the southern provenances. According to the results it seems to be possible to use growth characteristics as an indicator of frost hardiness at the provenance level.

Tutkimuksessa vertaillaan eteläistä ja pohjoista alkuperää olevien 1-vuotiaiden kuusentaimien kylmänkestävyyden ja rungon puutumisen kehitystä. Taimia kasvatettiin muovihuoneessa 10 viikkoa, jonka jälkeen +8 °C:n yölämpötilan ja +15 °C:n päivälämpötilan sekä asteittaisen yön pidentämisen avulla aloitettiin taimien talveentumisprosessi. Kylmänkestävyys määritettiin visuaalisesti neulasvaurioista, jotka syntyivät kun taimet pakastettiin –10 °C:n tai –15 °C:n lämpötilassa. Ennen pakastusta taimista mitattiin pituus, lignifikoituneen rungon osuus ja havainnoitiin silmun muodostuminen. Neulasvauriot kasvoivat alkuperän eteläisyyden myötä ja puutumattoman rungon osuus korreloi selvästi alkuperän kanssa. Yli 90 %:ssa pohjoisinta alkuperää olevissa taimissa oli silmu, vastaavan osuuden ollessa eteläisimmän alkuperän taimissa alle 1 %:n. Vaikka korrelaatio puutumattoman rungon osuuden ja neulasvaurioiden välillä oli korkea, korrelaatiokerroin kuitenkin vaihteli kaikkein pohjoisimman materiaalin 0,3:sta eteläisten erien yli 0,6:een. Tulosten perusteella näyttää olevan mahdollista käyttää kasvutunnuksia kylmänkestävyyden indikaattoreina ainakin provenienssitasolla.

Keywords: frost resistance, stems, lignification, seedlings, provenance, *Picea abies*.
FDC 181.2 + 161 + 181.5

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1 Introduction

The area of Norway spruce (*Picea abies* (L.) Karst.) cultivation in Finland has increased rapidly in recent years. To produce seed for cultivation 29 spruce seed orchard (total area about 300 ha) were established in Finland during 1957–1972. Six of the orchards have been established in southern and central Finland (1100–1200 d.d.) with clones originating from northern Finland (700–990 d.d.). Although most of the spruce seed orchards are now over 20 years old, the first and in fact the only good seed crop was harvested in 1989.

Utilizing the seed is, however, problematic because there is insufficient information available about background pollination. The origin and utilization area must be notified (Laki...1979, Maa- ja metsätalousministeriön...1987). To fulfill these requirements it is essential to know the background pollination level. This can be estimated by means of the isozyme technique and paternity analysis (Smith and Adams 1983, Friedman and Adams 1985), at least when the number of clones in the seed orchard is rather small. Furthermore, it is important to know the actual level of frost hardiness of the material produced in seed orchards established with northern clones. Because field tests with spruce take a long time early tests might provide the necessary information within a relatively short time period.

The aim of this study is to analyze differences between Norway spruce provenances by using artificial freezing test and especially some growth characteristics. The relationships between results from the artificial freezing test and some growth characteristics is also studied. Finally, the effectiveness of artificial freezing test and growth characteristics for evaluating crops of seed orchard established with northern clones is discussed.

2 Material and methods

The tested material consisted of seeds from four natural spruce stands located in areas ranging from southern to northern Finland (Table 1).

The seeds were sown in October 1990 in a greenhouse at Pieksämäki nursery. The plants were grown in vapo-blocks, without separate pots for each plant. Each block consisted of two different seed lots with 48 seedlings each. Each seed lot was replicated 18 times, thus the number of seedlings of each provenance was 864 and the total number of seedlings in the trial 3456.

The vapo-blocks were pre-fertilized and a growth fertilizer (Kekkilä 9, N 19.4 %, P 5.3 %, K 20.0 % and micronutrients) was applied during the growing period. During the freezing period a low-nitrogen fertilizer (Kekkilä 5, N 10.9 %, P 4.0 %, K 25.3 % and micronutrients) was used to keep the low nutrient levels stabilized. The seedlings were watered once or twice a week.

The mean day and night temperatures during the 10-week growing period were about 22 °C and 18 °C, respectively, with a 20-hour photoperiod and 50–190 $\mu Em^{-2}s^{-1}$ photon flux density provided by artificial lighting (Lightline RS 400 W SON XLT). During this time the temperature sum reached about 1060 d.d. After the 10-week growing period, the seedlings were hardened by lowering the day and night temperatures to +15 °C and +8 °C, respectively, and by increasing the night length by 1.5 hours a week until it reached 13 hours.

Frost hardiness of the seedlings was estimated by means of artificial freezing treatment, followed by visual needle injury assessment. The first block was frozen 24 days after the beginning of the hardening treatment, and the last one 40 day later. All of the nine freezing experiments (blocks) were carried out at both –10 °C and at –15 °C. One day before the freezing treatment the height, proportion of the lignified part of the stem and the bud-set of all seedlings were recorded. After the measurements the seedlings were exposed to +5 °C for 12 hours and the temperature was then decreased to –10 °C or –15 °C and maintained for four hours. The average freezing and warming rates were 3 °C $hour^{-1}$. After the freezing treatment the seedlings were transferred to the greenhouse. About two weeks later the extent of needle injury on the upper part (2 cm) of each seedling was assessed visually using seven classes as follows:

0 = no visual injuries
1 = < 20 % of needles injured
2 = 20–40 % of needles injured
3 = 40–60 % of needles injured
4 = 60–80 % of needles injured
5 = > 80 % of needles injured
6 = completely dead seedling

The injury values of seedlings were converted to percentages (ie. 0 = 0 %, 1 = 10 %, 2 = 30 %, 3 = 50 % etc.) and the mean values for the figures were calculated from these individual percentage values. In order to obtain samples for the statistical analyses that followed as closely as possible the normal distribution, the arcsin square-root transformation was done in the single seedling level. These transformed values of needle injury and the green proportion of the stem were used in all the statistical analyses.

The position of the seedlings within the box had no statistically significant effect on the growth or degree of needle injury of the seedlings. Neither did the position × provenance and position × block interactions. Because the position effect was almost nil, the studied model was reduced to following formula:

$$Y_{ijk} = \mu + P_i + R_j + PR_{ij} + e_{ijk}$$

where Y_{ijk} = mean needle injury or the green proportion of the stem in seedling
μ = total mean
P_i = effect of provenance
R_j = effect of block (the duration of the hardening treatment)
PR_{ij} = interaction between provenance and block
e_{ijk} = error

Two-way analysis of variance with the Tukey test was used to test the differences between provenances and blocks. Regression analysis was employed for detecting the relationships between the tested characteristics. The Spearman rank correlation analysis was also used.

Table 1. Origin of the tested Norway spruce provenances.

Origin	Year	Latitude	Longitude	Altitude, m	d.d.[1)]
Korpilahti	1989	62°13'	25°26'	200	1150
Sotkamo	1981	64°00'	28°32'	200	990
Rovaniemi	1989	66°15'	26°17'	150	840
Kittilä	1989	67°15'	24°24'	200	710

[1)] Threshold value is +5 °C. Based on the average values from Solantie (1976).

3 Results

The height, proportion of the green part of the stem and mean degree of needle injury decreased significantly from the provenances of southern origin to the more northern ones (Table 2). Furthermore, 81–92 % of the seedlings from the two northernmost provenances had visually detectable buds, but only 1–4 % of the seedlings of the two more southern provenances carried buds during the experiment (Table 2).

The proportions of green stem were significantly higher in seedlings from the two southernmost (Korpilahti, Sotkamo) provenances than in seedlings from northern (Rovaniemi, Kittilä) ones (Table 2, Fig. 1). The effect of provenance and also the effect of provenance × replication were significant (Table 3). In the seedlings from Rovaniemi and Kittilä provenances the green part proportion varied only from 9 % to 0 % (Fig. 1) during the hardening treatment period. However, the green part proportions in the seedlings of Korpilahti and Sotkamo provenances decreased sharply after 30 days hardening. At this point the difference between these two southern and two northern provenances was the greatest, being over 30 % (Fig. 1). During the hardening process the green part proportion decreased rather linearly from about 35 % to 8–13 % in the seedlings from Sotkamo and Korpilahti provenances.

The development of frost hardiness assessed on the basis of the mean degree of needle injury was also different between the provenances (Fig. 2ab). The provenance × block (length of hardening treatment) interaction had also a significant effect on the variation in mean needle injury (Table 3). During the first three freezing treatments the mean needle injury of Rovaniemi seed-

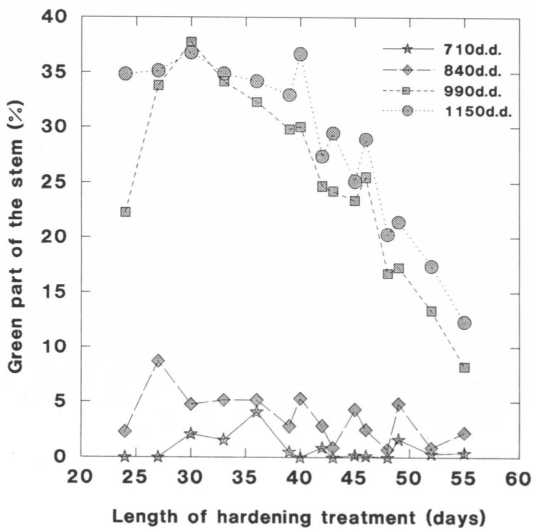

Fig. 1. Development of the mean proportion of the green, non-lignified stem in 1-year-old Norway spruce seedlings.

Table 2. Mean and standard deviation (SD) of the height, green proportion of stem, mean needle injury and proportion of seedlings with buds in 1-year-old Norway spruce seedlings.

Provenance	Height (mm)			Gps[1] (%)			Needle injury (%)			Buds (%)		
	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n
Korpilahti	22.0	31.0	663	28.2	10.5	663	62.7	35.3	839	0.7	8.4	839
Sotkamo	104.0	27.1	650	25.1	11.5	650	47.6	37.0	818	4.2	20.0	818
Rovaniemi	53.3	14.5	592	3.7	8.7	592	12.0	21.4	729	80.9	39.1	729
Kittilä	38.9	15.9	508	0.9	4.3	508	7.8	13.8	633	92.4	26.5	633

[1] Non-lignified, green proportion of the stem.

Table 3. Analysis of variance of mean needle injury and the proportion of green stem in 1-year-old Norway spruce seedlings.

Source of variation	Mean needle injury				Gps[1]			
	df	MS	F-ratio	p-value	df	MS	F-ratio	p-value
Provenance	3	95.75	1338.3	< 0.001	3	39.53	2465.6	< 0.001
Block	17	7.00	109.1	< 0.001	4	0.57	35.3	< 0.001
Provenance × block	51	1.69	23.6	< 0.001	42	0.12	7.4	< 0.001
Error	2947	0.07			947	0.02		

[1] Non-lignified, green proportion of the stem.

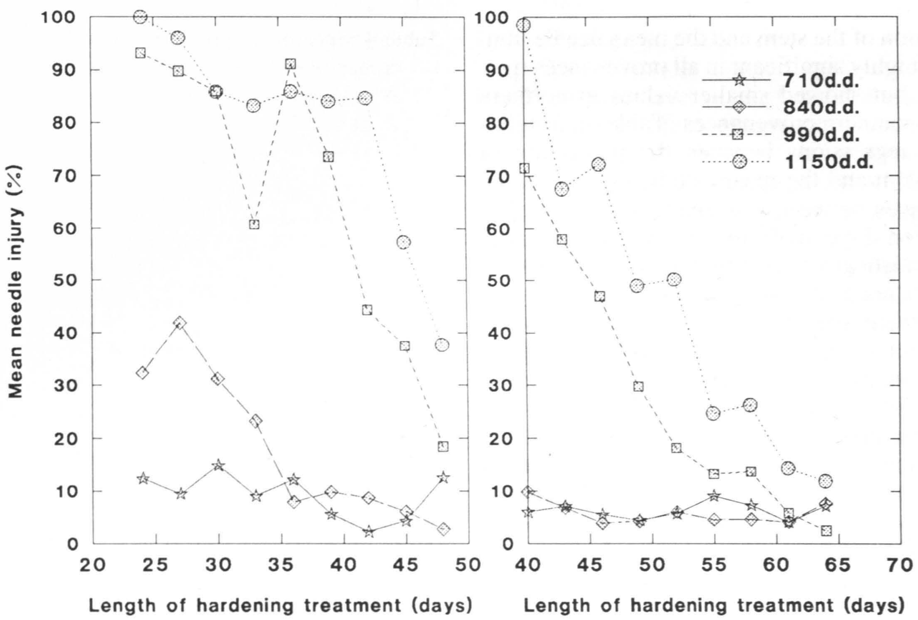

Fig. 2. Development of the mean needle injury in 1-year-old Norway spruce seedlings frozen at –10 °C (a) and –15 °C (b).

lings was significantly higher than in Kittilä seedlings (Fig. 2a), but decreased from 43 % to the same level as in the Kittilä provenance (about 10 %) after 36 days hardening and remained at the same level up until the end of the freezing treatments at –10 °C and –15 °C (Fig. 2ab). The Korpilahti and Sotkamo provenances had mean needle injury of over 90 % after 23 days hardening (Fig. 2a). The clear difference between these provenances and the provenances from Rovaniemi and Kittilä persisted to the end of the freezing treatment at –10 °C after 48 days hardening. The mean needle injury of the Sotkamo provenance reached the level of the Rovaniemi and Kittilä provenances after 62 days hardening (Fig. 2b). Furthermore, the mean injury level of the Korpilahti seedlings was significantly higher than that of the Sotkamo seedlings except in the case of freezing treatments carried out 30 and 36 days hardening (Fig. 2a). The variance model with provenance, block and interaction between provenance and block explained 72 % of the total variation in the mean degree of needle injury.

The overall correlations between the mean needle injury and height, the green proportion of the stem and the proportion of seedlings with buds were 0.61, 0.78 and –0.68, respectively. All these correlations were highly significant ($p < 0.001$). However, there were differences in the correla-

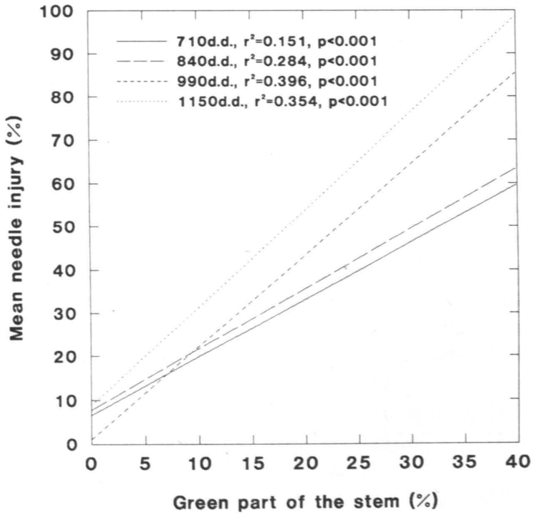

Fig. 3. Regression between mean needle injury and the proportion of the green, non-lignified stem in 1-year-old Norway spruce seedlings based on the values of individual seedlings.

tions between provenances (Table 4 and Fig. 3). Correlation between the mean needle injury and height was significant only in the Rovaniemi provenance. The correlation between the green proportion of the stem and the mean needle injury was highly significant in all provenances ($p < 0.001$), but showed smaller values in northern than in southern provenances (Table 4). Similarly, the regressions between the proportion of green stem and the mean needle injury showed differences between provenances (Fig. 3). The regression slopes of the two southern provenances were statistically higher ($p < 0.01$) than those of the two northern populations; the severity of injury increased more rapidly with increasing proportion of green stem in the two southern than in the two more northern provenances (Fig. 3). The low bud-set also indicated a high degree of needle injury (Table 4). The height and the proportion of green stem showed no correlation with the proportion of buds in the Korpilahti provenance, in contrast to the other provenances (Table 4).

Table 4. Spearman rank correlation coefficients and probabilities between the tested characteristics in different provenances of 1-year-old Norway spruce seedlings.

Provenance		Height	Gps[1)]	Injury[2)]
Korpilahti,	Gps	–0.03 NS		
N = 663	Injury	–0.01 NS	0.63 ***	
	Bud %	–0.06 NS	0.07 NS	–0.12 **
Sotkamo,	Gps	0.18 ***		
N = 650	Injury	0.08 NS	0.66 ***	
	Bud %	–0.21 ***	–0.18 ***	–0.17 ***
Rovaniemi,	Gps	0.53 ***		
N = 592	Injury	0.26 ***	0.45 ***	
	Bud %	–0.53 ***	–0.85 ***	–0.36 ***
Kittilä,	Gps	0.32 ***		
N = 507	Injury	–0.01 NS	0.32 ***	
	Bud %	–0.37 ***	–0.67 ***	–0.26 ***

1) Non-lignified, green proportion of the stem.
2) Needle injury.

4 Discussion

According to Johnsen (1989) the visual detection of needle damage is a suitable method for estimating freezing injuries in Norway spruce. However, the hardening treatment must be optimized in order to obtain as large variation as possible between provenances. The environmental conditions during growth and the hardening of the Scots pine seedlings, preceding the freezing tests, influence the results (Andersson 1992). It has been commonly stated that in Norway spruce the control of growth cessation is mainly based on photoperiod (e.g. Ekberg et al. 1979), and to a lesser extent on the temperature conditions. However, according Koski and Sievänen (1985) the temperature has also a major role for controlling the growth cessation. Owing to the relatively warm growing period and considerably mild hardening conditions, together with the small difference between the day and night temperatures during the hardening treatment, the differences in the development of frost hardiness between the southern and northern provenances were rather long when estimated in days. Large genetic variation in autumn frost hardiness has been noticed in many coniferous species (Rehfeld 1977, 1982, Johnsson et al. 1981, Cannell et al. 1985, Nilsson et al. 1991). Johnsen and Apeland (1988) found a high correlation in Norway spruce between freezing injury and terminal bud-set at the population level. The distinction between the provenances studied in the present study was clear in the case of bud-set: the two northern provenances had buds, the two southern ones did not, and the high bud-ratio was clearly associated with low mean needle injury values.

The material consisted of only four provenances, but even on the basis of this material the model, which included provenances, blocks (length of hardening treatment) and the interaction between these two factors, rather well explained the variation in the mean needle injury (see Table 3). This indicates the possibility of estimating the average frost hardiness of seed crops from seed orchards with an unknown proportion of background pollination by comparing the result of seed crops with the regression line of the mean needle injury of standard provenance material. This method is used e.g in Scots pine (e.g. Andersson and Westin 1990). The practical value of these estimations is, of course, strongly dependent on the correlations between estimated injury in artificial freezing tests and the real survival in the field. The correlations between survival in field tests and the degree of needle injury caused by artificial freezing treatment is quite high (Rehfeld 1977, Andersson 1986, Nilsson and Eriksson 1986, Nilsson and Andersson 1987, Nilsson et al. 1991). According to Andersson (1992) the correlation between early freezing damage in Scots pine seedlings and the field mortality after 10–12 years was positive and high for experiments at climatically severe localities, but weaker for field trials at milder sites with lower mortality.

The proportion of the non-lignified part of the stem (green part) seemed to correlate rather well with the degree of freezing injury, but the correlation tended to decrease with increasing latitude of the provenance. An overall negative correlation between height and freezing injury has been demonstrated earlier (Johnsen and Apeland 1988). According to the present results, this correlation was weak in the southernmost provenances (before bud-set).

Using an indirect method for assessing the frost hardiness level of seed orchard crops may be problematic. This is because the adaptive characters e.g bud-set did not appear to correlate with freezing injury at the family level (Skrøppa 1991). This might also be true for stem lignification.

The use of freezing tests for estimating the background pollination level in Norway spruce seed orchards may also be problematic, because there is considerable genetic variation in frost hardiness development between Norway spruce trees within the same stand (Skrøppa 1991). Secondly, the mating in Norway spruce and Scots pine seed orchards is not random, but strongly biased (Skrøppa and Tutturen 1985, Johnsson et al. 1976, Koski 1980). Thus the estimation of background pollination is also biased owing to the different frost hardiness development of the clones acting as pollen and/or seed parents.

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