Screening early autumn frost hardiness among progenies from Norway spruce seed orchards

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Nursery-grown Norway spruce (Picea abies (L.) Karst.) seedlings from 12 different seed orchards were tested for early autumn frost hardiness using artificial freezing tests. Seed orchards containing grafted parent clones originating from high altitudes produced seedlings showing higher damage than commercial control seed lots of the same altitudes. The low altitude material did not differ from the comparable commercial controls. A seed orchard containing both German and Norwegian clones produced seedlings showing high damage. The correlation between bud-set and frost damage was high at the provenance level, but lower at the half- and full-sib levels. Families with good growth capacity in progeny field tests showed large between-family variation in frost damage in the artificial freezing tests. This indicates the possibility to combine high growth rate with acceptable autumn frost hardiness in the selection of parent trees.

Keywords: Picea abies, progeny testing, bud-set, growth cessation, breeding

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1. Introduction

In Norway a future goal is to harvest a high percentage of Norway spruce seeds from genetically improved seed orchards. A large testing program involving several thousand parent trees is already in operation. The progeny tests consist of polycross families from first generation seed orchards, and open-pollinated families from standing trees in the forest. These are short-term tests (10–12 years) designed for backward selection of parent trees. Often, however, the genetic entries are not subjected to sufficient environmental stresses, such as early autumn frosts, during the short-term field tests. Artificial freezing tests may then be a good substi-

tute giving a quick assessment of autumn frost hardiness. Artificial freezing tests have shown good agreement with field performance both in general (Levitt 1980), and also specifically in conifers (Riehfeldt 1977, 1979a, Jonsson et al. 1981, Nilsson and Eriksson 1986).

The present paper reports results from artificial freezing tests on progenies from several Norway spruce seed orchards. Relationships between bud-set and frost damage, and between frost damage and field height growth are presented. Some possible implications for breeding are discussed.

2. Materials and methods

2.1 Plant material

The entire material comprised 84 separate seed lots. Bulked open-pollinated seeds harvested in 1983 from 12 different seed orchards and 11 different open-pollinated commercial seed lots collected in forest stands in zones B and C were used (Fig. 1). The seed lots from zone B were collected between 300–800 m. elevation (B3–B8), and the seed lots from zone C were between 100–500 metres (C1–C5). The seed orchards comprised grafted clones phenotypically selected in natural stands within zones B and C. The Roma seed orchard was established with both Norwegian and German clones, the latter were selected in older plantations in the southern part of Norway. This orchard was separated into two compartments and one bulked open-pollinated seed lot was collected from each of them.

Open-pollinated seeds were also collected from clones within three of the orchards. From the Kaupanger seed orchard 10 half-sib families were included. From Roma, 26 half-sib families were used, and from Svenneby, 17 half-sib families were included. Seven full-

sib families from pair-crosses between 14 unrelated parent clones located in Svenneby were also included. These 14 parent clones had high breeding values for field height growth.

Seeds were sown in April 1984 in a greenhouse at Gvarv nursery. The plants were grown in multipot containers in three replicates with 30 seedlings per seed lot and replicates. The seedlings were grown indoors until July, and then moved outdoors to our experimental farm at As, where they developed frost hardiness naturally outdoors during September, 1984.

2.2 Freezing tests

Due to limited space in the freezing chambers, the plant material was divided into two parts: material originating from medium to high elevation (part 1), and material from the lowlands (part 2). Part 1 was frozen to −10°C on 6 September and to −20°C on 19 September. Part 2 was tested one day after at the same two temperatures (7 and 20 September).

Tests were performed with eight plants from each replicate. Each genetic entry was placed in four freezing chambers so that two plants from each of three replicates were placed in each chamber. The roots were insulated by putting the multipot containers into containers made of foamed polystyrene and the space between the plants was filled with 1.5 cm of perlite. The freezing chambers were equipped with a circulating fan and were equally programmed to freeze at a constant rate from +5 to either −10 or −20°C within 6 hours. The freezing tests lasted 4 hours at −10 or −20°C and thawing was made to +5°C within six hours. Thus, the freezing and thawing rates were faster at −20°C. However, when comparing results from −10°C with results from −20°C, very stable ranking between experimental entries was found (Johnsen 1988).

2.3 Bud-set and needle damage assessment

The bud-set of each seedling was classified one day before the freezing tests according to the following:

0 = no terminal bud, needles light green and stem succulent at the top.
1 = tiny white terminal bud, needles light green and white lateral buds near the apex.
2 = terminal bud light brown (development of bud scales), the lateral buds light brown and needles rather light green.
3 = well developed bud scales, larger bud and dark green needles shortened at the apex.

After tests the plants were placed in a greenhouse at 20°C, under humid conditions and continuous light (supplementary light from Luma white fluorescence tubes, 38 W). Needle damage was scored after three weeks as follows:

0 = no visible damage, needles green.
1–10 = Classification of brown or decolorized needles in ten percent classes of needles exposed to frost.
11 = all needles completely brown, most plants entirely dead.

2.4 Statistical procedure

Analyses of variance were done both to obtain a reliable estimate of the experimental error, needed for comparison between means, and to test whether the variance component caused by the genetic entries was significant in both bud set and frost damage. Plots, R²-values, and rank correlations (RC) between
bud-set and frost damage at different genetic levels were only shown when the variation between means of genetic entries was significant in both traits.

Some problems must be solved when analysing frost damage and bud-set. The variables are not continuous and deviate from the normal distribution (Johnsen 1988). In addition, those entries that have expectations near the extreme values 0 or 11 for needle damage, and 0 or 3 for bud-set, have lower variances than the others. However, the transformation used by Norell et al. (1986) taking into account both normalization and homogenization of variance between groups could be used (Johnsen 1988).

The mean of 8 plants per combination of genetic entry, replicate, and temperature was used as the unit of observation. This variable was transformed to arsine (s/11)² for needle damage, and to arsine (s/3)² for bud-set.

The analyses of variance were performed with the following model:

\[ X_{ik} = u + G_i + R_k + t + G R_{ik} + e_{ik} \]

where

- \( X_{ik} \) = the observation (frost damage means, bud-set means)
- \( u \) = total mean
- \( G_i \) = the fixed or random effect of genetic entry \( i \)
- \( R_k \) = the random effect of replicate \( j \), \( j = 1, 2, 3 \)
- \( t \) = the fixed effect of test temperature \( k \), \( k = 1, 2, 3, 4 \)
- \( G R_{ik} \) = the interaction between genetic entry and replicate
- \( e_{ik} \) = residuals

2.5 Field trials

The half-sib families from Svenneby and Kaupanger seed orchards were also included in progeny tests. The tests were located at two sites, Behle and Biri, both at an elevation of 550 metres. Seeds were sown in 1977, the plants were grown in mutipot containers for two years and then planted in the two field trials during the spring of 1979. Height was measured in 1984, and the mean height performance from the two sites was expressed in percentages of the total mean.

Fig. 2. Mean needle damage scores of bulked open-pollinated seed lots from orchards and open-pollinated commercial seed lots. The letters B and C refer to the seed collection zones in Fig. 1. Numbers associated with B, C, and the orchard names, indicates the altitude of commercial seed collection and the altitudinal range of clones in orchards in 100 m. elevation. The different shadings indicates part 1 (upper bars) and part 2 of the material.

3. Results

Commercial and bulked open-pollinated materials were analysed and compared for possible differences in mean needle damage (Fig. 2). Seed orchards with clonal material from 600 to 800 metres (Kaupanger, Jensberg) produced progenies with significantly more damages than the comparable controls from the same elevations (B6–B8, \( p = 0.01 \)).

Opsahl seed orchard produced the hardiest progenies compared to the other orchards. The plants were only slightly though significantly more damaged than the mean of the controls B5 to B8 (\( p = 0.02 \)). The orchards containing clones predominantly from medium elevations (Svenneby, Romedal and Drogseth) should be compared with both C

The lowland orchards (Eley, Maystad, Huse and Stange) produced seedlings with the same hardness level as the C- controls from 100–300 metres. However, both compartments of the Romes seed orchard produced seedlings with significantly more damage (\( p = 0.01 \)) than all other low elevation material tested.

The commercial provenances and bulked open-pollinated seed lots from the orchards showed a high correlation between bud-set and frost damage (Figs. 3 a,b). Lower, but still rather high correlations were found when half-sib families from a compartment of Romes and from Kaupanger were analysed (Figs. 3 c,d). Most progenies from German mothers had delayed bud-set and high damage. Half-sib families from the Svenneby orchard showed a lowering correlation (Fig. 3 e). Families that had different mean bud-set values often had similar frost hardness levels, and families with similar mean bud-set values often had different hardness levels. The correlation between bud-set and frost damage was weak and non-significant when full-sib families were analysed (Fig. 3 f). Five of the full-sib families showed large variation in bud-set but only minor differences in frost damage.

An examination of the relationships between frost damage in artificial environment and height growth in the field for half-sib families from Svenneby seed orchard (Fig. 4) indicates that although all grew well compared to the controls, the families showed wide variation in frost damage. However, some families combined good growth with acceptable hardness. Similar relationship was found for half-sib families from the Kaupanger seed orchard (Fig. 5). Good growers from the field tests were highly variable in frost damage, but only two of the ten families combined good growth with acceptable hardness compared to the controls.
4. Discussion

4.1 Hardiness of bulked seed from the orchards

The seed orchards containing clones with origins from the highest altitudes (Kaupanger, Jønsberg and Opsahl) produced plants that were less hardy than comparable controls B6–B8 (Fig. 2). Jønsberg and Opsahl are located at 200 m. elevation and are exposed to natural pollen from the surrounding forest. Thus, pollen contamination may account for reduced hardiness in these orchards.

Kaupanger seed orchard, however, is very well isolated from outside pollen. It is located at 25 m. elevation in western Norway, over 100 km away from the nearest natural stand of Norway spruce, and at the inner part of a large fjord surrounded by high mountains (Fig. 1). Both female and male flowering have been very abundant for several years in this orchard (Skreppe and Tutturren 1985), and it is thus unlikely that outside pollen could contribute significantly. However, the altitudinal transfer of clones has been large (from 600–800 down to 25 metres), leading to a very different parental environment during flowering, fertilization and seed ripening compared to that of the controls. Whether changes in parent plant environment due to altitudinal transfer will affect the progeny performance, as indicated by Rowe (1964), remains to be tested in future investigations.

Pollen contamination could account for a slight reduction in hardiness in progenies from the orchards containing clones from 200–600 metres i.e. Svenneby, Romdal and Drogsedt (Fig. 2). Reduced hardiness is less important in these orchards than in the orchards giving plants to be used at the highest altitudes. In order to avoid risks, seedlings from the Romdal seed orchard should not be planted on severe sites at 500 m. elevation.

At the moment, it is difficult to determine where to use the bulked seed from the Gvay seed orchard. It is located at 20 m. elevation and contains clones from 300–800 metres, a large altitudinal span. In addition, the or-
chard is surrounded by native Norway spruce adapted to the warm climate at Gvarv. Thus, it is likely that pollen contamination will reduce the hardiness level of progenies from this orchard.

The hardiness of seedlings from the lowland orchards, Eløy, Mestad, Huse and Stange (Fig. 2), was equal to that of the comparable controls (C1-C3). However, seedlings from both compartments of Romsa seed orchard produced seedlings with high damage scores. This orchard was established with both Norwegian and German clones. The German mother trees produced offspring with delayed bud-set and high freezing damage (Fig. 3c). It is likely that progenies from the German mothers reduced the hardiness level of the bulked seed from this orchard. Seeds from Romsa could be used within zones with a mild climate such as western and southern Norway. However, Romsa is located on a site unfavorable for flowering (Skreppa and Tutturren 1985), and thus the practical usefulness of this orchard is unclear.

4.2 Bud-set and frost hardiness

Both commercial provenances and the bulked seed lots from the orchards showed a high correlation between bud-set and frost damage (Figs. 3 a,b). Several parent trees corresponded to each mean value and a large altitudinal range was included as well as latitudinal differences since Romsa was included. Ecotypic differences have been found in growth cessation at the rovenance or population level in several conifer species (Heide 1974, Eriksson et al. 1978, Rehfelt 1979b, 1983, 1986, Mikola, 1982). The same has been found for autumn frost hardiness (Rehfelt 1977, 1980, 1982, 1986, Sandvik 1980, Jonsson et al. 1981, Carmell et al. 1985, Anderson 1986, Nilsson and Eriksson 1986). The general trend is that populations from marginal areas (high altitudes or latitudes) tend to have an early growth cessation and an early development of frost hardiness. Thus, at the provenance or population level, a close relationship between bud-set and autumn frost hardiness should be expected. In a compartment at Romsa (Romsa 2), the latitudinal difference between the trees is reflected in both bud-set and frost damage (Fig. 3c). The German mothers (open symbols) produced offspring with late bud-set and large damage values; the total variation in one trait (height) does not necessarily imply a decrease in variation of other traits (bud-set and frost hardiness). This is based on the genetic correlations between traits which may be high or low depending on the traits in question (Dean et al. 1983). Genetic correlation between growth cessation and autumn frost hardiness has not been studied in Norway spruce.

Information about the relationship between bud-set and autumn frost hardiness is scarce in conifers. The correlation has been found to be fairly high at the population level in Douglas-fir (Rehfelt 1979b, 1983). To my knowledge, this paper is the first publication dealing with the relationship between bud-set and autumn frost hardiness at the family level with Norway spruce. However, more information will be published in the near future. A frequency distribution with a tendency toward half-sib families from diallel crosses within two stands of Norway spruce, revealed low non-significant correlations between bud-set and frost damage, even though families with large differences in bud-set were included in the tests (Skreppa 1988).

The present results clearly indicate that good correlation between growth cessation and frost hardiness at the provenance level (Fig. 3a) is also found at other genetic levels, e.g. half-sib or full-sib families (Figs. 3 c,e). When screening at the provenance level, bud-set can predict differences in autumn frost hardiness, but within orchards (between half-sib or full-sib families), direct measurement of frost hardiness is necessary. In a study with black spruce, Dietrichson (1969) found a high correlation between lignification and winter frost damage at the provenance level ($R^2 = 0.948$), but a low correlation at the family level ($R^2 = 0.147$). Thus, lignification could only predict winter damage at the provenance level and not at the family level. This supports the practical conclusion that direct selection according to frost hardiness is better than an indirect selection according to differences in growth rhythm.

4.3 Frost hardness and field height performance

Correlation between height and frost damage (Fig. 4) indicates that selection for height in field test can be combined with acceptable hardiness in freezing tests. This relationship also illustrates that it is important to understand more that one trait when backward selection is used for establishment of genetically improved seed orchards. The less hardy combiners should be used in another plantation zone (perhaps regrafted in another seed orchard). Alternatively, they could be used on favourable sites (southern slopes) within the intended zone. The most frost susceptible combiners could also be culled from the planting population if a reasonable number of trees with high breeding values for both traits are available.

The corresponding plot for Kaupanger seed orchard shows that only two of the ten mother trees combine good growth with acceptable hardiness compared to relevant controls (Fig. 5). In seed orchards meant to provide seeds for marginal areas like Kaupanger, the most important trait must be frost hardiness. Thus growth performance should have lower priority. The hardness level of both the bulked material (Fig. 2) and the well-growing half-sib families from Kaupanger (Fig. 5) is low, and the hardness should be improved by selecting the parent trees giving the hardest progenies in this orchard.

4.4 Limitation of the freezing tests

Freezing tests reported in this paper will be included in the progeny test program for Norway spruce in Norway. However, some few limitations should be pointed out. Due to limited space in the freezing chambers, only a single temperature can be included per test. Neither variation within seed lots nor any estimates of actual lethal temperatures (Gjerum 1985) can be given from the variation obtained using a single temperature.

The tests reported in this paper were conducted with nursery-grown seedlings during
cold acclimation in September. Tests at −10 °C in the beginning of September and −20 °C the third week of September are very severe, and there is a risk of overheating the significance of the results, at least for genetic materials to be used at low elevation in the southern or south-eastern parts of Norway. However, tests like those reported in this paper will provide information for culling the most susceptible parent trees by either not including them in the breeding population, or using them in another population covering a zone less exposed to frequent early autumn frosts.

References


Variation in the rate of winter hardening of one-year-old plus-tree families of Scots pine raised in different environments

Jan-Erik Nilsson

THIVSTELMÅ: YMPÄRISTÖTIEKÖIDEN VAikutUS YKSIVUOTTAIDEN MÄNNyn PLuSuPPUJÄKELÄIStÖ-JEN TALVENTUMISNOPEUTEE


The effects of different environmental conditions (four outdoor localities and one greenhouse locality in northern Sweden) on cold hardening of 29 one-year-old full-sib families from full-sib trees of Scots pine were studied by artificial freeze testing. Plants exposed to low night temperatures during August achieved faster cold hardening than plants raised in milder localities. The family ranking for rate of winter hardening was consistent among outdoor localities if freeze testing was performed at times when plants from different localities had attained similar levels of cold hardness. However, significant family x locality interactions were obtained when plants from the outdoor localities were freeze tested on the same occasion. Freezing damage was positively correlated with plant height but not correlated with dry matter content in the autumn. Freezing damage of greenhouse raised plus-tree families was uncorrelated with damage of plants raised outdoor. Possible implications for hardness breeding are suggested.

Ympäristötieköiden vaikutusta 29 yksivuotiaan männyn pluspuuläkkälistöjen talventumisnopeuteen

Keywords: cold acclimation, freeze testing, full-sibs, seed orchard, selection

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