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# Performance in early tests, short-term trials and field trials of selected families of Norway spruce from Svenneby Seed Orchard

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Divisjon for skog og utmark/Avdeling for skoggenetikk og biomangfold

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Performance in early tests, short-term trials and field trials of selected families of Norway spruce from Svenneby Seed Orchard

Resultater fra tidlige tester, korttidforsøk og feltforsøk med utvalgte familier av gran fra Svenneby frøplantasje

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**SUMMARY/SAMMENDRAG:**

Norway spruce parents selected from results in progeny trials for testing Svenneby Seed Orchard clones were crossed in a factorial crossing design, and full-sib families were planted in short-term and field trials in southern Norway. Artificial freezing tests with the same families were made the first growing season and at ages 10 and 12 years. Offspring of parents selected for superior growth were taller in the field tests than those from parents with heights lower than the mean in the progeny test. However, the correlations between half-sib family performance in the initial progeny test and in the offspring field tests were only moderate. The variation among families in injury scores in the freezing tests was large, but the relationships with field trial performance were weak, indicating that frost hardiness testing at a young age is not valuable for predicting later field performance. Families with a late timing of bud flush had the highest frequency of injuries after a frost event at mid-summer. These families were also the tallest in the field tests. The families from crosses among Svenneby Seed Orchard clones had better height growth than commercial provenances.

Foreldretrær av gran, valgt ut fra resultater i avkomforsøk for å teste kloner i Svenneby frøplantasje, ble krysset etter en faktoriell krysningsplan, og full-søsken familiene ble plantet i korttidforsøk og i langsiktige feltforsøk. Fryseforsøk med familiene ble gjort etter første vekstsesong og ved alder 10 og

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12 år. Avkom fra foreldre valgt ut for god høydevekst var høyere i feltforsøkene enn de fra foreldre som ble valgt ut med midlere høyde. Det var bare moderate korrelasjoner mellom høyder til halv-søsken i de første avkomforsøkene og i feltforsøkene. Det var betydelig variasjon mellom familiene i skader etter frysforsøkene, men sammenhengene var svake mellom frostskaider og vekst og skader i feltforsøkene. Dette indikerer at resultater fra testing av frostherdighet i ung alder sier lite om hvordan trær fra familiene senere vokser i felt. Familier med sen skuddskyting fikk mest skader etter frostnetter 22.-23. juni. De sent skytende familiene hadde best høydevekst i feltforsøkene. Trærne fra familiene fra Svenneby frøplantasje var høyere enn de fra hadde handelsprovenienser.

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# 1 Introduction

Breeding of Norway spruce in Norway was initiated by selection of trees with superior height growth and outstanding form and branching traits in natural stands. Grafted seed orchards were established based on clones of the selected trees. It was soon recognised that because most of the forest occupy very variable sites and were often uneven-aged, much of the phenotypic variation of the selected trees were under environmental control. Progeny trials to estimate the genetic value of the clones in the seed orchards were necessary. Such trials should be planted a several sites. They were also important for providing information about the genetic variation of important traits and their relationships.

Svenneby seed orchard (latitude 60°37', longitude 11°54', altitude 155 m) was established in the early 1960's with 175 clones from altitudes 325-620 m in southern-eastern Norway and with the aim of producing seeds for use at altitudes 350-550 m. Controlled crosses were made in the orchard in 1976 to produce seed lots for progeny testing and two trials were planted. Eleven years later new crosses were made among selected parents based on the results from the progeny tests, and a series of new trials were established.

This report presents results from the initial progeny trials, from freezing tests made at different ages and from trials planted at several sites with offspring from the selected parents and with seedlings from commercial provenances. It provides information about the genetic variation and inheritance patterns of the offspring of the selected parents and of relationships between traits observed at different ages and in trials at several sites.

## 2 Materials and methods

### 2.1 Initial progeny trials and selection of parents for crosses

Progeny trials were planted in 1979 at two sites (Bøle, Veggli, altitude 550 m and Enga, Biri, altitude 520 m) with 110 half-sib families after controlled crosses made at Svenneby Seed Orchard with a pollen mixture from 15 male parents. The parental clones originated from altitudes between 325 and 620 m. The trials were planted at former agricultural soil, at 0.8 m spacing, and with random distribution of one seedling of each family (single tree plot) in 20 blocks. In addition, seedlings from commercial provenances (B3, A4, B4, C4, A5, B5, C5, A6, B6) (Skogfrøverket 1995) were planted. The height of the trees was measured regularly, and in 1986, ten years from seed, two sets each of eight parents were selected. The clones in one set had offspring with superior height growth and those in the other had family height means lower than the total mean across both trials. In each of the sets, four of the clones were chosen to be either the maternal (mother) or the paternal parent (father) in a uncomplete factorial design in controlled crosses made at Svenneby Seed Orchard in 1987, illustrated in Table 1. This factorial design contained three groups of crosses defined by the mean height growth of the offspring of the parents, 1: good x good; 2: good x poor and 3: poor x poor.

**Table 1. Controlled crosses producing 48 full-sib families. Parents 1 to 4 had offspring with superior height growth and parents 5 to 8 had off-spring with height growth below the mean of the two progeny trials.**

Maternal parent	Paternal parent							
	1	2	3	4	5	6	7	8
1	X	X	X	X				
2	X	X	X	X				
3	X	X	X	X				
4	X	X	X	X				
5	X	X	X	X	X	X	X	X
6	X	X	X	X	X	X	X	X
7	X	X	X	X	X	X	X	X
8	X	X	X	X	X	X	X	X

### 2.2 Trials with full-sib families

In the spring of 1988 seeds from the 48 full-sib families and from three control provenances (B3, B4 and B5) were germinated in the nursery at Hogsmark Experimental Farm, Ås, and seedlings were cultivated with standard cultivation methods for two growing seasons before they in 1990 were planted in one short-term trial on agricultural soil (Nilsrud) and three long-term field trials (Bamsrud, Veldre sag, Tørberget) (Table 2). The short-term trial was planted at 1 m spacing and with 40 blocks of single-tree plots. The field trials were planted at 2 m spacing and with 30 blocks.

A second sowing with the same family seed lots were made in the nursery at Hogsmark in 1989, and seedlings were cultivated in the greenhouse following standard routines for fertilizing and watering (e. g. Bjørnstad 1981) until they were moved outdoors in early August to be acclimated. In the spring of



1990, seedlings from these families were planted in a short-term trial (Hogsmark) and two field trials (Lindbo, Løvli) (Table 2).

**Table 2. Short-term and long-term field trials planted 1990. Mortality and height refer to mean values at the last measurement.**

<b>Trials</b>	<b>Latitude</b>	<b>Altitude m</b>	<b>Last year of measurements</b>	<b>Mortality %</b>	<b>Height cm</b>
<b>Short-term:</b>					
<b>Hogsmark, Ås</b>	59°40′	85	1999	2.9	246
<b>Nilsrud, Kongsvinger</b>	60°10′	190	2001	3.3	422
<b>Long-term:</b>					
<b>Bamserud, Etnedal</b>	60°52′	625	2004	8.9	354
<b>Veldre sag, Veldre</b>	60°57′	520	2003	46.7	256
<b>Tørberget, Trysil</b>	61°08′	520	2004	54.7	334
<b>Løvli, Løten</b>	60°53′	350	2018	35.6	923
<b>Lindbo, Sigdal</b>	60°00′	500	1992	35.6	

Artificial freezing tests with seedlings from the 48 full-sib families were made in Mid-September 1989, at the end of the first growing season. These tests were made in four freezing chambers at minimum temperatures of -13°C following procedures described by Johnsen (1989) and Johnsen et al. (1996). After freezing, the seedlings were placed in a greenhouse under humid conditions and freezing injuries on needles were assessed after three weeks on individual plants in classes 0-11: 0=no visible damage, 1-10=ten percent intervals of brown or discoloured needles, 11=all needles completely brown.

In the spring of 1999, at the beginning of growing season 11, twigs were collected from the third and fourth whorl of trees in the short-term trial at Hogsmark. They were frozen on April 21 in 10 freezing chambers each with a minimum temperature between -8 to -32°C. Maximal variation among the full-sib families was obtained at temperatures between -17 and -23 °C, and these three test temperatures were chosen for the analysis of injury scores. A similar freezing test was performed on October 1, 2000 (age 12 years), with minimum temperatures between -12 to -33°C. Here, maximal variation among families was obtained at temperatures between -15 to -33°C. After the freezing tests, the twigs were kept for three weeks at continuous light and 20 –24 °C in a misting chamber where they developed freezing injury symptoms (browning). In both tests, needle injury was classified visually on individual twigs according to the classes used in the 1989 freezing test.

## 2.3 Measurements

Tree heights were measured at irregular intervals in the trials. Here, analyses will be made of heights measured at ages 11 years from seed (1999) at Hogsmark, 14 years (2001) at Nilsrud, 16 years (2003) at Veldre sag, 17 years (2004) at Bamserud and Tørberget and 17 (2004) and 30 (2018) years at Løvli. The last year diameter was also measured. In the short-term trial at Hogsmark assessments of bud flush were made in the spring of 1999 based on the Krutzsch scale (Krutzsch 1973). Assessments were made in some trials of injuries after frost events and of stem damage (double leaders, ramicorn branches) when heights were measured.

## 2.4 Statistical analyses

Traits assessed in classes were transformed to normal scores within blocks before statistical analyses were made. Injury data from the freezing tests of twigs were transformed to normal scores within each test temperature and replicate (Gianola and Norton 1981; Falconer and Mackey 1996). Negative values represent hardier and positive values less hardy seedlings than average. Analyses of variance were made based on the transformed values. Pearson correlation coefficients were calculated between mean

values of full-sib families for traits measured in different tests or trials, ignoring half-sib family relationships. Analyses of variance were made of individual tree heights measured in 1986 in the two progeny trials at Enga and Bøle with families and blocks within trials as random effects in the model. Similar analysis was made of heights measured in the period 1999 to 2004 in the four trials Hogsmark, Nilsrud, Bamsrud and Løvli by a model containing fixed effects of the family crossing groups and random effects of mother and father nested within groups and their interaction, interactions with trials and random effects of blocks within trials. Estimates of heritability of height were made based on the variance components estimated. Correlation coefficients were calculated for parental family means for height 1986 in the initial progeny test, against maternal and paternal half-sib family means within Groups 1 and 2, and 2 and 3, respectively, in the four trials described above. Relationships between field test performance and freezing test and bud flush scores were characterized by correlation coefficients at the full-sib family level.

Statistical analyses were made in SAS (SAS Institute 2003).



## 3 Results

### 3.1 Initial progeny trials and selection of parents

The mortality in 1986, eight years after planting, was 18.4 and 1.4 % and mean heights were 123 and 190 cm at Enga and Bøle, respectively. In the analysis of variance of height, significant variation ( $p=0.001$ ) was present both between half-sib families and for the interaction between families and site ( $p=0.002$ ). The correlation coefficient between family mean heights in the two trials was as low as  $r=0.30$  and the estimate of heritability for height was 0.11. Across the two sites the family mean heights varied from 80 to 120 % of the total mean. The provenances from 250–450 m (B3, A4, B4, C4) had a mean height of 95 and those from 450–650 m (A5, B5, C5, A6, B6) 92 % of the total mean height of the two trials. The mean height of the families from the two selected groups of parents were 114 and 97 % of the total mean.

### 3.2 Freezing tests of one-year-old seedlings

The mean injury scores for the seedlings were for the three crossing groups 5.3, 5.3 and 5.5, respectively, showing a slightly higher level of injury for the families in Group 3 (poor x poor). Significant differences were present both for mother and fathers ( $p<0.0001$ ) within groups and no mother x father interaction.

### 3.3 Freezing test of twigs

There was a large variation among families in normal score values of freezing injuries in both freezing trials and significant variation ( $p=0.001$ ) among the three family groups (Table 3). Group 1 (good x good) was the hardier in the spring freezing test and least hardy in the autumn test (Table 3).

**Table 3.** Mean values for crossing groups of damage scores in the early freezing test, of freezing injuries in spring and autumn at Hogsmark in normal score units and for bud flush scores. Negative score values represent hardier and positive values lesser hardy seedlings than average. The three crossing groups are defined as 1: good x good; 2: poor x good; 3: poor x poor.

Trait	Crossing group		
	1	2	3
Freezing injury year 1	5.3	5.3	5.5
Freezing injury spring 1999	-0.22	-0.07	0.27
Freezing injury autumn 2000	0.11	-0.06	-0.13
Bud flush 1996	2.6	2.5	3.0

### 3.4 Variation in short-term trials

The mean mortality was low both at Hogsmark and Nilsrud (Table 2) and with variation among the full-sib families from 0 to 10 %. For height in 1999 (Hogsmark) and 2001 (Nilsrud), Group 2 had the highest values and group 3 the lowest (Table 4). In the analysis of variance these differences were not significant. However, significant variation was present both among mothers and fathers within groups ( $p<0.004$ ). The range of variation in bud flush among full-sib families was from 2.6 to 3.7 units, which corresponds to approximately six days, and the mean values of the groups were 2.6, 2.5 and 3.0 (Table 3). Significant variation ( $p<0.05$ ) was present both among groups, and among mothers and fathers within groups.

### 3.5 Variation in field trials

The high mortality in four of the field trials was due to severe frost events that took place on June 22 and 23 1992, two years after planting. At Veldre sag and Tørberget, severe injuries occurred, but no assessments were made. At Lindbo, 36 % of the plants were classified as dead and 25 % as severely injured, with 62, 68 and 56 % of the dead or severely injured in the groups 1, 2 and 3, respectively. This trial was then terminated. At Bamserud, 35 % of the plants were injured, with a variation among families from 17 to 57 %, and with the lowest mean (31 %) for Group 3. Twenty percent of the plants died or were damaged at Løvliå, and the mortality increased in 2018 to 30, 37 and 40 %, respectively, in the three groups. At the other sites, there were at the last measurement minor differences in mortality among the crossing groups. At Tørberget, 35 % of the surviving trees had stem defects in 2004, with small differences among the groups and large differences among families within groups. In 2018 at Løvliå, 33 % of the trees were assessed with more than one stem defect since 2004, with range of variation from 11 to 75 % among full-sib families and small differences among the crossing groups.

The mean height of Group 3 was the lowest at all sites (Table 4), and Group 1 had the highest mean in three of the field trials. In the analysis of variance of height across the four trials Hogsmark, Nilsrud, Bamserud and Løvliå, measured in the period 1999 to 2004, no significant effects of crossing group nor interaction between groups and trials were present ( $p > 0.30$ ). Main reasons for the lack of significance among the groups were that the offspring of two of the mothers and one of the fathers in Group 1 showed poor height growth and that the offspring of one of the mothers in Groups 2 and 3 showed superior growth. Significant differences were present between mothers and fathers within groups, for their interactions and for interactions between parents and sites ( $p < 0.001$ ). The last interactions were chiefly due to weak correspondence between the family mean heights at Bamserud and the other three trials. The correlation coefficient between the mean heights of the full-sib families among these trials were in the range 0.51 to 0.62.

**Table 4. Mean values of height (cm) and diameter (mm) for crossing groups at all trials. The three crossing groups are defined as 1: good x good; 2: poor x good; 3: poor x poor. The last column shows the mean height of the provenances B3, B4 and B6 at the four sites where they were planted.**

Trait, year and trial	Crossing group			B3, B4, B5
	1	2	3	
Height 1999 Hogsmark	254	259	238	
Height 2001 Nilsrud	429	436	400	341
Height 2003 Bamserud	361	362	338	285
Height 2003 Veldre sag	266	260	242	236
Height 2004 Tørberget	363	339	298	309
Height 2004 Løvliå	352	335	315	
Height 2018 Løvliå	965	926	897	
Diameter 2018 Løvliå	115	107	103	

There were minor differences in mortality among the provenances B3, B4 and B5, and the mean height of B5 was 13 % lower than the mean of B3 and B4. The mean mortality of the three provenances was equal to that of the families at Nilsrud and Bamserud, and 6 to 10 % higher at Veldre sag and Tørberget. At the two first sites, the families were on average 24 % taller than the provenances, and 8 % taller at Veldre sag and Tørberget (Table 4).

### 3.6 Correlation patterns

No significant relationship was present at the full-sib family level between the injury score in the early freezing test made in the autumn the first year and the injury score of the twigs frozen in the autumn of growing season 12 ( $r=0.08$ ). A weak positive relationship ( $r=0.32$ ,  $p=0.03$ ) was found between the first year's freezing test scores and the injury scores in the freezing test of twigs made in the spring of growing season 11. No relationships were present between the injury scores in the spring and first autumn freezing tests with twigs ( $r=0.11$ ).

The timing of bud flush assessed at Hogsmark showed no relationship with the scores of injuries observed after the autumn freezing tests but had a positive correlation with the injury scores on the twigs frozen in the early spring of growing season 11 ( $r=0.60$ ,  $p<0.001$ ). Thus, the highest injury scores occurred on the twigs of trees that later had an early bud flush.

There were no significant relationships between the injury scores at Bamserud in 1992 and those from the freezing tests. However, a significant correlation ( $r=0.39$ ,  $p=0.008$ ) was present between the percentage of trees not injured and the timing of bud flush at Hogsmark as trees from families with an early bud flush was least injured. A similar effect was observed at Lindbo where there the correlation coefficient between the percentage of dead plants and bud flush was  $r=-0.61$  ( $p=0.001$ ).

In the trial at Løvlia, bud flush was positively correlated with the percentage of trees with stem and top damage in 2001 ( $r=0.42$ ); families with an early bud flush had a higher frequency of trees with damage. At the other sites, no significant relationships were found between the percentages of trees with stem damage in the field trials and the injury scores from the freezing tests nor for the timing of bud flush.

At the full-sib family level there were significant correlation coefficients between tree heights up to age 16 years and both bud flush and injury scores in the spring freezing test, varying in the range from -0.38 to -0.52. However, at Løvlia this correlation was reduced from -0.39 at age 16 years to -0.16 at age 30 years. Families with an early bud flush had the lowest mean heights.

No relationship was found between mean height 1999 to 2004 in four trials and percentage mean height in the initial progeny test for maternal half-sib families in Groups 1 and 2 ( $r=-0.02$ ). For the paternal half-sib families in Group 2 and 3, the relationship was stronger ( $r=0.65$ ). If the correlation coefficients were estimated separately from the trial at Bøle, then the values were  $r=0.32$  and  $r=0.75$ , respectively.

## 4 Discussion

In the initial progeny tests planted in 1979, several of the families did not rank similarly for height at age eight years at the two sites from which the selection was made of parents for new crosses, and a family x site interaction was expressed. Since the selection of parents were based on the mean performance across the two sites this interaction may have influenced the selection efficiency and reduced the expected differences in performance among full-sib family Groups 1, 2 and 3 in the offspring generation (Table 3). The altitudes of the two sites are in the recommended zone for the use of plant materials from Svenneby seed orchard, and no information is available about environmental factors at Enga and Bøle that could have caused this interaction. Two other progeny test series were established at the same two sites and were measured the same years. In these trials, there were no family x site interactions for height (Skrøppa, unpublished).

The main effect of the selection of parents was that Group 3 had the lowest mean height at all sites (Table 3). Minor differences were present between Groups 1 and 2. The differences in relationship between mean progeny heights 1999 to 2004 at four sites and progeny test performance at Enga and Bøle questions the relevance of the Enga site progeny test for a successful selection of parents for the next generation. In conclusion, selection of parents for further breeding should be based on progeny tests at several sites and factors behind family times sites interactions should be examined.

The trees from the commercial provenances had similar mean heights as those from Group 3 at the two sites with high mortality after the frost events in 1992 (Veldre sag and Tørberget). At the two other sites their heights were inferior. Lower height growth of trees from commercial provenances than of those from seed orchard seeds was earlier demonstrated by Kohmann (2003) and Skrøppa et al. (2005).

In the freezing test at the end of the first growing season, minor variation was present among the three groups of families, but differences were present in the freezing test of twigs at ages 10 and 12 years. At these ages, the trees in Group 3 with the lowest tree heights in the trials were on average more frost hardy in the autumn, and less frost hardy in the early spring. The last result corresponds with the differences observed in the timing of bud flush for the groups and is the same for the full-sib families.

The large variation observed among the families in frost hardiness at the end of the first growing season corresponds to what has been demonstrated in other tests of families from seed orchards, i. e. Johnsen and Apeland (1988), Johnsen and Skrøppa (1992). The lack of relationship between young and adolescent frost hardiness clearly shows that frost hardiness in early tests is not a good predictor of autumn frost hardiness of adolescent trees. Similar results have been shown in other trials by Johnsen (unpublished) and Skrøppa (unpublished). The study also shows a lack of relationship between the freezing test results and mortality and stem damage in the field trials. Our data thus indicate that results from frost hardiness testing of families at a young age cannot predict their performance in the field at high altitudes.

The mid-summer frost event in 1992 caused the highest frequency of injuries to the late flushing families. This contrasts to what has been observed in trials on sites at lower altitudes exposed to late spring frosts, where the early flushing families have been most injured (Hannerz et al. 1999; Berlin et al 2015). Most likely the trees with an early bud flush were in mid-summer at a development stage less vulnerable to low temperatures. It has been shown that frost hardiness varies during the shoot elongation period (Repo 1992).

In all trials there was a significant negative correlation between the timing of bud flush and height growth, and late flushing families were usually the tallest. This corresponds with results from studies by Hannerz et al. (1999) and Skrøppa and Steffenrem (2016) who found negative genetic correlations between the timing of bud flush and height growth. Thus, a late bud flush is beneficial both for avoiding injuries by spring frost events and for better height growth.

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Norsk institutt for bioøkonomi (NIBIO) ble opprettet 1. juli 2015 som en fusjon av Bioforsk, Norsk institutt for landbruksøkonomisk forskning (NILF) og Norsk institutt for skog og landskap.

Bioøkonomi baserer seg på utnyttelse og forvaltning av biologiske ressurser fra jord og hav, fremfor en fossil økonomi som er basert på kull, olje og gass. NIBIO skal være nasjonalt ledende for utvikling av kunnskap om bioøkonomi.

Gjennom forskning og kunnskapsproduksjon skal instituttet bidra til matsikkerhet, bærekraftig ressursforvaltning, innovasjon og verdiskaping innenfor verdikjedene for mat, skog og andre biobaserte næringer. Instituttet skal levere forskning, forvaltningsstøtte og kunnskap til anvendelse i nasjonal beredskap, forvaltning, næringsliv og samfunnet for øvrig.

NIBIO er eid av Landbruks- og matdepartementet som et forvaltningsorgan med særskilte fullmakter og eget styre. Hovedkontoret er på Ås. Instituttet har flere regionale enheter og et avdelingskontor i Oslo.