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Weak relationships between injuries in freezing tests and performance in short-term and field trials of Norway spruce families from Stange Seed Orchard

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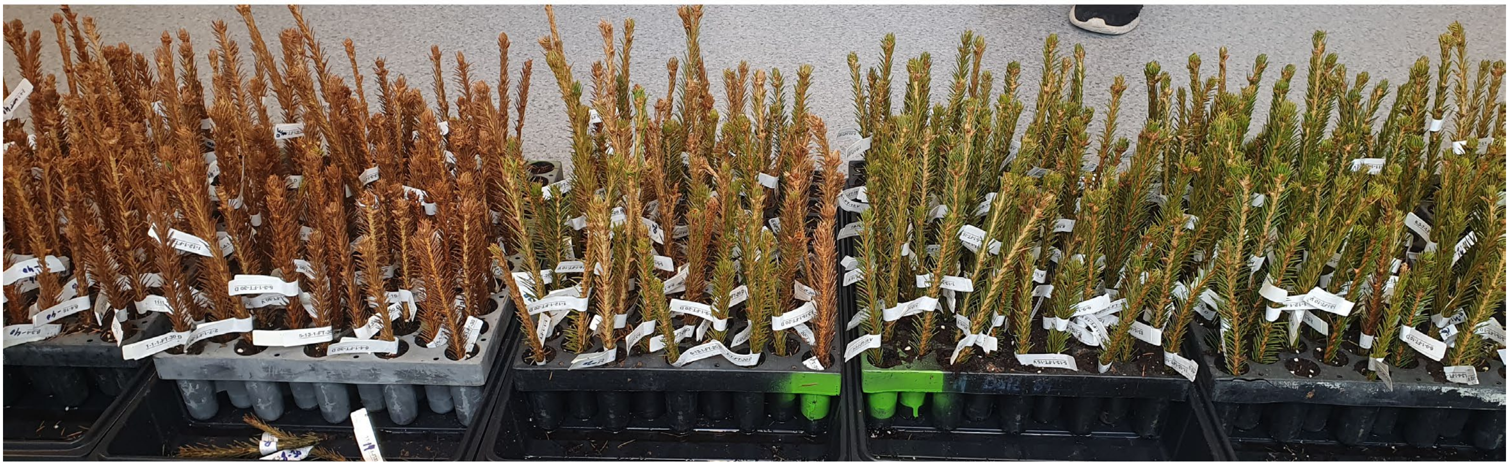

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SUMMARY/SAMMENDRAG:

Artificial freezing tests were performed on seedlings from Norway spruce families at the end of the first growing season. Similar tests were made on twigs collected from trees in a progeny test at the end of growing season nine. The 26 families in the early test were included in the short-term progeny test with 100 full-sib families from a 10 x 10 factorial cross. All families were also planted in seven field trials in Norway, Sweden and Finland, from which data on mortality, tree heights and stem damage at age 10 years are available. Significant difference was found among families for freezing test injuries on whole intact seedlings at the end of the first growing season and for lethal temperature of needles on detached twigs collected at the end of growing season nine. However, no relationships were found between the freezing test scores of families in the two types of tests or few between these scores and the traits measured in the short-term and field trials. The results show that frost hardiness testing of families at a young age, grown under artificial temperature and light conditions in nursery, is a weak predictor of their performance under natural conditions in field at older ages.

Fryseforsøk ble utført med granplanter ved slutten av første vekstsesong og med skudd fra de samme familiene samlet inn fra et avkomforsøk som var ni år gammelt. Familiene i fryseforsøket var en del

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av et sett med 100 full-søsken familier som alle ble plantet i syv feltforsøk i Norge, Sverige og Finland. Fra disse forsøkene er data tilgjengelig for avgang, høyde og skader etter 10 år. Det var signifikant forskjell mellom familier for fryseskader på ettårige planter og for letal temperatur for nåler på kvistene samlet inn ved ni års alder. For familiene var det imidlertid ingen sammenheng mellom frostskader i de to fryseforsøkene eller mellom frostskader og egenskaper målt i kortidsforsøk eller i feltforsøkene. Forsøkene viser at resultater fra fryseforsøk i ung alder, der plantene er dyrket under kunstige temperatur og lysforhold, ikke nødvendigvis gir en god prediksjon for overlevelse, vekst og skader til familier etter flere år i feltforsøk under naturlige forhold.

LAND/COUNTRY:	Norway

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Tor Myking	Arne Steffenrem
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Content

1 Introduction

High survival and avoidance of frost damage are important for the success of Norway spruce plantations at an early age. A proper choice of materials is therefore crucial. Many studies have demonstrated genetic variation in autumn-, winter- and spring frost hardiness both at the population (Dietrichson 1969; Skrøppa & Dietrichson 1986) and family level (Hannerz 1994; Hannerz *et al.* 1999). Often frost hardiness can be related to the timing of the annual growth rhythm of the material and the climatic conditions at the specific site (Dietrichson 1969; Skrøppa & Steffenrem 2016). A screening of autumn frost hardiness can be made in artificial freezing tests of seedlings at the end of the first growing season, and several studies have shown genetic variation in frost hardiness among provenances and families in such tests (Johnsen & Apeland 1988; Johnsen 1989; Skrøppa 1991, Skrøppa *et al.* 1994¸ Dæhlen *et al.* 1995). For freezing tests to be efficient for prediction field performance and be useful for selection in tree breeding programs there must be a relationship between hardiness under freezing test conditions and performance under field conditions.

This study reports relationships between injuries on 26 Norway spruce families after artificial freezing tests at the end of the first growing season and the performance of the same families in two short-term tests and during ten years in field trials at seven sites in three Nordic countries. It also presents the variation in hardiness of needles of nine-year-old trees and relationships between such injuries and performance of families in short-term and field trials.

2 Material and methods

2.1 Trials and measurements

A provenance hybrid factorial cross in Norway spruce was performed at Stange Seed Orchard (lat. 60°40′, alt. 180 m) between 10 parents of Norwegian (N) and 10 parents of East-European (E) origin (Skrøppa and Steffenrem 2016). The N parents were selected trees from natural stands in southern Norway and the E parents were selected plus trees in a 25-year-old provenance trial in northern Sweden. They were all grafted at Stange Seed Orchard. Five parents from each provenance group were used as female and five as male parents. The family groups with only Norwegian and only East-European parents will be referred to as N x N and E x E families, respectively.

Twenty-six full-sib families from the factorial cross were included in an artificial freezing test made at the end of the first growing period of seedlings grown in the Phytotron, The University of Oslo (Johnsen & Skrøppa 1992). One day before freezing, bud set was recorded on individual plants in classes 0 – 3: 0=no terminal bud, 1=tiny white terminal bud, 2=well developed terminal bud light brown, 3=well developed bud scales. Freezing injuries on needles were assessed on individual plant three weeks after freezing in classes 0-11; 0=no visible damage, 1-10 = ten percent intervals of brown or discoloured needles; 11= all needles completely brown.

In 1985, two short-term trials were established on cultivated soil with the 100 families from the factorial cross (Skrøppa & Steffenrem 2016). One trial was planted at Hogsmark Experimental Farm, Ås, Norway (lat. 59°40′, alt. 50 m) and the other at Trysil Forest Nursery (lat. 61°18, alt. 360 m), located approximately 200 km northeast of Ås. The experimental design in both trials was randomised complete blocks with 12 blocks and four-tree family plots with 1 m spacing. Heights of individual trees were measured in the experiment at Hogsmark after five (1989) and eight (1992) growing seasons. Similar measurements were made at Trysil at the end of the fifth growing season. At this site, a large proportion of the trees were damaged during the late autumn of 1988 and winter of 1989 and assessments of damage were made in the spring of 1989. In the early summer of 1990, late spring frost occurred at Trysil, and new assessments of spring damage were made. At Hogsmark, the elongation of terminal shoots was measured weekly during the growing season in 1989 and 1990, and estimates were made of the days of initiation and cessation of shoot growth (Skrøppa and Steffenrem 2016).

The 100 full-sib families were planted in field trials at seven sites in 1988 in Norway, Sweden, and Finland under very diverse climatic conditions (Skrøppa & Steffenrem 2021). Mortality, tree heights and assessment data of ramicorn branches, double stems and forks are available from these trials.

In September 1993, twigs collected in the short-term trial at Hogsmark were freeze tested. From all trees in six blocks, 40 twigs were detached from lateral shoots of branches selected in the same whorl. Twigs for testing were taken from two blocks the first week, another two blocks the second week and two more blocks the third week of September. Twigs were put in plastic bags and cold stored for one day before being frozen in freezing chambers. Four twigs from each tree were placed in four-tree plots in trays filled with wet peat, distributed in a completely random design. Thus, ten groups of trays were available for each test, one to be placed in each of ten freezing chambers. The chambers were programmed to minimum temperature levels -5, -7, …., - 21, -23 degrees during the first freezing test and 2 and 4 degrees lower on the next two freezing occasions. After the freezing test, the twigs were placed under humid conditions for three weeks and assessments of freezing injuries on needles were made in the same classes as was used for the freezing test with seedlings described above.

2.2 Statistical analyses

Traits assessed in classes were transformed to normal scores within blocks before the statistical analyses. Mean score values are presented. Similarly, percentages were transformed by the arcsine transformation before the statistical analyses.

For each tree, a non-linear relationship was estimated between temperature level and the mean transformed injury score of its four twigs at that level. Estimates were then made of the temperatures when 50 % (LT50) and 70 % (LT70) of the needles were brown or discoloured. Analyses of variance were made of these temperatures by the models presented by Skrøppa & Steffenrem (2016, 2021), adding a fixed effect of the freezing events and its interaction with the parents. An estimate of the narrow sense heritability was calculated for LT50.

Pearson correlation coefficients were calculated between mean injury values of the 26 full-sib families in the early freezing test and traits measured in the short-term and field trials. Similar calculations were made for all 100 families between LT50 means and the traits measured in both types of trials and for the 20 half-sib family means. These calculations were made both for trait values in each individual field trials and for means across all trials. Family relationships were ignored in these analyses.

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

3 Results

3.1 Freezing test of one-year-old seedlings

The variation in frost injury score means of the 26 full-sib families was from 2.1 to 9.0 and in terminal bud-set from 0.2 to 3.0 (Johnsen & Skrøppa 1992). For both traits highly significant variation was present among families ($p<0.0001$). The families from East-European parents had a slightly later bud set and had an injury score one unit higher than those of Norwegian parents. The correlation coefficient at the full-sib family level between the two traits was $r=-0.87$; the families with a late bud set had more severe frost injuries.

3.2 Variation in short-term and field tests

Significant additive genetic variation was present among families within the N x N and E x E groups for tree heights and the days of shoot growth initiation and cessation in the trial at Hogsmark (Skrøppa & Steffenrem 2016). Mean tree heights were similar for the N x N and E x E family groups, but those in the last group had the latest start and cessation of shoot elongation. At Trysil, significant variation was also present for the percentage of trees with winter frost injuries in 1989 and spring frost injuries in 1990, the last year with significantly higher injury frequencies for the N x N families than for the other family groups.

In the field trials, the mean mortality and the percentage of trees with stem defects varied among the sites and among families within sites. For tree heights at age ten years, significant additive genetic variances were present, and some family by site interactions were present with small variance components values (Skrøppa & Steffenrem 2021).

3.3 Freezing tests with twigs

Small and non-significant variation was present among the crossing groups for LT50 (N x N: -15.2, E x E: -14.5 degrees) and LT70 (N x N: - 16.6, E x E: -15.9 degrees). The range of variation among the full-sib families was from -9.6 to -19.7 degrees (Table 1). In the GLM analysis of variance of LT50 of all families pooled, variance components were significant both among female and male parents, but not for the female-male interaction. This indicates additive genetic variation for this trait. No interactions were present between parents and freezing events. The estimate of heritability for LT50 was $h^2=0.45$.

Table 1. Mean values of LT50 at age 9 of the 100 full-sib families in the factorial cross. All degrees have negative values. Maternal parents are listed in the left column and paternal parents in the upper row.

Parent	11	12	13	14	15	16	17	18	19	20	Mean
1	15.4	15.7	17.2	14.5	14.2	14.4	15.5	14.4	14.1	14.3	15.0
2	12.4	14.3	14.5	13.0	13.6	13.5	12.7	11.7	11.2	11.9	12.9
3	15.1	17.8	18.1	13.5	17.7	15.5	17.8	13.8	16.6	16.3	16.2
4	12.6	14.2	14.6	12.5	13.1	11.2	13.2	9.6	12.0	11.0	12.4
5	15.6	18.1	19.7	16.5	16.2	14.6	18.5	15.5	16.3	15.5	16.6
6	14.1	17.8	17.6	15.0	14.3	14.4	16.6	14.3	14.4	15.2	15.4
7	15.5	17.1	17.6	14.9	13.7	14.6	16.7	14.0	14.4	14.8	15.3
8	14.1	16.4	14.6	13.3	13.4	13.5	16.6	12.3	13.3	13.6	14.1
9	13.4	13.8	14.8	13.0	13.4	13.9	14.6	14.0	14.3	12.4	13.8
10	13.7	15.1	16.4	14.3	15.4	14.1	16.0	14.3	16.5	13.2	14.9
Mean	14.2	16.0	16.5	14.1	14.5	14.0	15.8	13.4	14.3	13.8	14.7

3.4 Trait relationships

Few of the phenotypic correlation coefficients that were estimated between the injury scores of the 26 full-sib families in the first-year's freezing test and traits measured in the short-term and field trials indicated strong relationships (Table 2). Only two correlations coefficients were clearly significant: the day of shoot growth cessation at Hogsmark (r=0.61, p=0.001) and mortality at the field trial at Imatra, Finland (r=0.43, p=0.03). Two field trials had a high mortality after three and four years (43 and 21 %), but no significant correlations were present between the family means for mortality in the field and freezing test injuries. The correlation coefficient between bud set and the day of shoot growth cessation at Hogsmark age 5-6 was r=-0.46 (not shown), showing that families with an early bud set the first growing season also ceased shoot growth early five and six years later. At three of the field trial sites, families with a high injury score were among those with the tallest heights at age ten years (not shown). The correlation coefficient between the (transformed) injury scores after hardiness testing the first year and LT50 had a low value (r=-0.19).

Table 2. Estimated phenotypic correlation coefficients between the injury scores in the first-year's freezing test and traits measured in the short-term and field trials of the 26 full-sib families. Absolute values of estimates higher than 0.40 (bold) can be considered significant at the 5 % level.

Trait	Trial	Estimate of correlation coefficient
Height age 8	Hogsmark	0.37
Shoot growth initiation	Hogsmark	0.31
Shoot growth cessation	Hogsmark	0.61
Winter frost damage	Trysil	0.18
Spring frost damage	Trysil	0.16
LT50	Hogsmark	-0.19
Mortality	Imatra	0.43
Mean mortality	Seven field trials	0.12
Mean height age 10	Seven field trials	0.29
Mean stem defect	Seven field trials	-0.17

Phenotypic correlation coefficients between LT50 if twigs and traits measured in the short-term and field trials were estimated both based on the 100 full-sib families (not shown) and on the 20 half-sib families. For half-sib families there were no significant relationships between LT50 and any of the traits measured in the two short-term trials or mean values across the seven field trials (Table 3). This was the case also for the full-sib families.

Table 3. Estimated phenotypic correlation coefficients of 20 half-sib family means between the lethal temperature LT50 and traits measured in the short-term and field trials. Absolute values of estimates higher than 0.46 can be considered significant at the 5 % level.

Trait	Trial	Estimate of correlation coefficient
Height age 8	Hogsmark	-0.15
Shoot growth initiation	Hogsmark	-0.13
Shoot growth cessation	Hogsmark	-0.17
Winter frost injury	Trysil	-0.03
Spring frost injury	Trysil	-0.12
Mean mortality age 10	Seven field trials	-0.33
Mean height age 10	Seven field trials	-0.17
Mean stem damage	Seven field trials	-0.26

4 Discussion

Significant variation was found among families for freezing test injuries on whole intact seedlings at the end of the first growing season and for lethal temperature of needles on detached twigs collected at the end of growing season nine. Both methods have been widely used to measure hardiness (e. g. Johnsen 1989; Aitken et al. 1996; Beuker et al. 1998). When the humidity is high under misting conditions, browning of injured tissue on twigs develop as in intact seedlings, and it is expected that the method satisfactorily measures the hardiness of the trees at the time of shoot collection.

The family variation found for lethal temperature at the end of growing season nine corresponds with what was observed in similar freezing tests with families from Svenneby Seed Orchard (Skrøppa and Steffenrem 2022). In the present trial, a high value of heritability was estimated for LT50 (r=0.45). In Douglas fir, heritability estimates between 0.09 and 0.22 were found for autumn cold hardiness traits on nine-year-old trees (Aitken et al. 1996). The sites of field trials represent a wide range of environmental variation in the Nordic countries, from latitude 57°47' to 64°17', with large climatic differences among sites. There was also large variation in mean mortality, from 24 to 40 %, and for percent trees with stem defects from 8 to 37 % among these trials (Skrøppa and Steffenrem 2021).

The seedlings tested after the first growing season were grown under a regime that is common for nursery testing, with continuous light for a period before cold and long night acclimatisation (Johnsen and Skrøppa 1992). At this age, it is practically impossible to grow the seedlings under natural conditions regarding germination, watering, temperature, light, and photoperiod. Under natural conditions, the seedlings would have finalized growth and developed bud much earlier, often at a size of 0.5 – 2 cm (height), while in nursery they have a prolonged growing season and reach a size of 10 – 30 cm.

A major finding in this work is the lack of relationship observed between the freezing test traits in the two types of tests and between these scores and the traits measured in the short-term and field trials. Similar lack of relationship between young and adolescent frost hardiness was reported by Skrøppa and Steffenrem (2022). Here, the only significant correlation coefficient was between hardiness at the end of the first growing season, and the timing of shoot growth cessation year five and six, and mortality at one of the field trials: families with a high injury score (low hardiness) had a late shoot growth cessation five years later and higher mortality at the Imatra at age 10. There was no consistent relationship between the freezing test traits and tree heights in the two types of trials, similarly to what was found for Douglas fir by Aitken et al. (1996).

These results confirm the statement by Skrøppa and Steffenrem (2022) that frost hardiness testing of families at a young age, grown under artificial temperature and light conditions, cannot predict the performance in the field at older ages. The poor correlation between young and adolescent stages indicates that frost hardiness is not the same genetic trait at the two ages, possibly interacted by the different growing conditions and physiology of the plants. This can be partly explained by the changes in growth patterns that take place (Ununger et al. 1988). The first-year seedlings exhibit exclusively free growth and needle primordia elongate shortly after they have been formed. From the second year and beyond, an increasing part of the annual shoots is predetermined since the amount of annual shoot growth is determined the preceding year by the number of needle primordia in the overwintered bud. This changes the growth rhythm traits and also influences frost hardiness.

In field, mortality and damages to needles, apical and cambial meristems can be induced by many different abiotic and biotic factors. Many of these might be uncorrelated with the hardiness in fall tested here. Even frost injuries, such as those occurring in spring is not necessarily correlated to hardiness in fall. Hence, traits related to frost injuries will be influenced by an array of modifying factors that hardly can be simulated under controlled growing conditions. Thus, in breeding and for development of deployment recommendations for assisted migration, long-term testing in the field is a necessity. Short-term tests under controlled conditions cannot substitute the field testing across climate gradients within and among the breeding zones.

Freezing tests of nursery seedlings might still have an important role to safeguard the quality of the plants that are used in regenerations. This is particularly important in relation to the quality after storage, but also related to production of new seed sources that might have different production requirements in the nursery.

Freezing test of twigs from older trees might be important as indicator of the spring and fall hardiness of seed sources that are relevant for long-distance assisted migration. It is then important to test the adaptive traits most important for the tree's health, development and resistance to secondary pathogens. However, this testing cannot substitute field testing and evaluation of the trees' accumulated growth and vitality over many years under natural conditions.

References

Aitken, S.N., Adams W.T., Schermann, N, & Fuchigami L.H. 1996. Family variation in fall cold hardiness in two Washington populations of coastal Douglas fir (Pseudotsuga menziesii var. Menziesii (Mirb.) Franco. For. Ecol. Manage 80:187-195. For. Ecol. Manage 80:187-195.

Beuker, E., Valtonen, E. & Repo, T. 1998. Seasonal variation in the frost hardiness of Scots pine and Norway spruce in old provenance experiments. For. Ecol. Mange. 107:87-98.

Dietrichson, J. 1969. The geographic variation of spring-frost resistance and growth cessation in Norway spruce (Picea abies (L.) Karst.). Medd norske SkogforsVes. 27:91-106.

Dæhlen, A.G., Johnsen, Ø. & Kohmann, K. 1995. Høstfrostherdigheten hos unge granplanter fra hanndelsprovenienser og frøplantasjer. Rapport fra Skogforsk 1/95:1-24.

Johnsen, Ø. 1989. Freeze testing young Picea abies plants. A methodological study. Scand J. For. Res. 4:351-367.

Johnsen, Ø. & Apeland, I. 1988. Screening early autumn frost hardiness among progenies from Norway spruce seed orchards. Silva Fennica 22:203-212.

Johnsen, Ø. & Skrøppa, T. 1992. Possible influence of natural and artificial selection on autumn frost hardiness in Picea abies. Meddelelser fra Norsk institutt for skogforskning 45.1:1-12.

Hannerz, M. 1994. Damage to Norway spruce (Picea abies (L.) Karst.) seedlings caused by late spring frost. Skogforsk Report No.5 1994: 1-19.

Hannerz, M. & Sonesson, J. 1994.Genetic correlations between growth and growth rhythm observed in a short-term test and performance in long-term field trials of Norway spruce.

Skrøppa, T. 1991. Within-population variation in autumn frost hardiness and its relationship to bud-set and height growth in Picea abies. Scand. J. For. Res. 6:353-363.

Skrøppa, T. & Dietrichson, J. 1986. Winter damage in the IUFRO 1964/68 provenance experiment with Norway spruce (Picea abies (L.) Karst.). Meddelelser fra Norsk institutt for skogforskning 39.10:161-184.

Skrøppa, T., Nikkanen, T., Routsalainen, S. & Johnsen Ø. 1994. Effects of sexual reproduction at different latitudes on performance of the progeny of Picea abies. Silvae Genetica 43:298-304.

Skrøppa, T. & Steffenrem, A. 2016. Selection in a provenance trial of Norway spruce (Picea abies (L.) Karst.) produced a land race with desirable properties. Scand. J. For. Res. 31:439-449.

Skrøppa, T. & Steffenrem, A. 2021. Performance and phenotypic stability of Norway spruce provenances, families, and clones growing under diverse climatic conditions in four Nordic countries. Forests 2021, 12, 230.

Skrøppa, T. & Steffenrem, A. 2022. Performance in early tests, short-term trials and field trials of selected families of Norway spruce from Svenneby Seed Orchard. NIBIO Rapport 8/87/2022: 1-13.

Ununger, J., Ekberg, I. & Kang, H. 1988. Genetic control and age-related changes of juvenile growth characters in Picea abies. Scand. J. For. Res. 3:55-66.

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NIBIO is owned by the Ministry of Agriculture and Food as an administrative agency with special authorization and its own board. The main office is located at Ås. The Institute has several regional divisions and a branch office in Oslo.

Cover photo: Arne Steffenrem (field trial) and Ishita Ahuja (freezing test).

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