Variation Among Open-pollinated Families of *Picea abies* (L.) KARST. in Response to Simulated Frost Desiccation

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1. Abstract

Vitality, height growth, biomass, phenology and morphology traits of 36 open-pollinated families of *Picea abies* were studied in growth chambers after frost desiccation treatment performed during the later part of the rest period after the 1st and the 2nd growth periods. Frost desiccation was simulated during 3 weeks by exposing the above-ground part of the seedlings having roots in a frozen substrate to relatively high temperature (15 °C/5 °C day/night) and by increasing the light intensity from 300 µmol/m²/s to 483 µmol/m²/s for 4 h in the middle of 12 h day. The underground part of the seedlings was maintained frozen by immersing the seedlings in freezing baths filled with glycol providing –3 °C for the roots. Frost desiccation treatment had significant effect on all the traits studied. Besides reduced leader elongation, the observable types of damage varied from death of the leader to death of whole seedling. Seedlings treated after the 2nd growth period were damaged more than seedlings treated after the 1st growth period. Family effect on the frost desiccation damage score was significant. The genetic correlation between the family damage scores in the two treatments was significant. Seedling height and bud flushing were strongly genetically correlated with the frost desiccation damage score. Early flushing families containing relatively short seedlings were less damaged than late flushing families containing relatively tall seedlings. The tolerance to frost desiccation is attributable to a high degree of hardiness possessed by early flushing and short seedlings. Breeding for tolerance to frost desiccation would be efficient after prior selection of provenance.

Key words: Picea abies, frost desiccation, damage, genetic variation, phenology, growth traits.

2. Introduction

In late winter an intensive sun irradiation may cause desiccation in shoots of Norway spruce seedlings having roots in frozen ground. The bright sunshine increases temperature during the day which causes transpiration out of the foliage while water supply is prevented. A drop of the water content below the critical level may occur in seedlings being exposed for a long period to such conditions which would result in irreversible damage. This damage is referred to as frost desiccation or frost drought (TRANQUILLINI, 1982), a phenomenon that was reported for *Pinus sylvestris* seedlings by EBERMAYER as early as in 1901. NEGER (1915) and MÜNCH (1933) debated that this damage may have been caused by frost rather than by desiccation. However, frost desiccation was shown to be the main determinant of the damage by SAKAI (1968, 1970) and MICHAEL (1963) under field conditions and by CHRISTERSSON et al. (1987) in growth chambers. We have experienced severe frost desiccation damage (>90%) on *Picea abies* seedlings in one of our nursery trials in early spring 1997. During a cloudless period of three weeks the temperature fluctuated from –10 °C to +10 °C.

Weather conditions causing frost desiccation of *Picea abies* seedlings grown on open areas occur in some years in the northern distribution area of *Picea abies* in late winter. Frost desiccation may therefore be a selective agent during such years in nature. This could have provoked genetic variation in tolerance to frost desiccation. DORMLING (1993) reported that a *Picea abies* population from Belarus was less tolerant to frost desiccation than a population from southern Sweden. According to HANNERZ (1994) *Picea abies* clones with late growth cessation tended to be more damaged by frost desiccation than clones ceasing growth early in a clonal seed orchard located in central Sweden. In agreement with this LARSEN (1978) reported a more slow desiccation in high elevation populations of *Pseudotsuga menziesii* than in low elevation populations. HATAKEYAMA (1981) in a study on frost desiccation damage within and among provenances of *Abies sachalinensis,* experienced a higher tolerance to frost desiccation of the provenances which originated from the areas with high incidence of frost desiccation. We are not aware of any study on genetic variation in tolerance to frost desiccation in *Picea abies.* Such studies are essential if tolerance to frost desiccation is included in a breeding programme.

Our objectives were to estimate genetic parameters for tolerance to frost desiccation under controlled conditions in a *Picea abies* population from a clonal seed orchard.

3. Material and Methods

3.1 Material and cultivation

Open-pollinated seeds were collected from 36 Norway spruce clones grown in the Maglehem seed orchard in southern Sweden (55°50'N, 14°07'E, 60 m a.s.l.). The clones were selected in southern Swedish stands (lat. 56° to 59°) which originated from continental Europe.

The experiment was performed in growth chambers for three growth periods. The frost desiccation treatments were carried out after the 1st (referred to treatment D1) and after the 2nd (treatment D2) growth periods. 540 seedlings (15 seedlings per family) were designated for each of the treatments and the control (referred to as C), altogether 1620 seedlings in the experiment. The experimental design was blocks with non-contiguous plots. One seedling per family was randomly allocated within a plot, i.e. 36 seedlings per plot. During the growth in the chambers, 5 blocks with 3 plots per block were used within each of the treatments.

The seedlings were grown in plastic tubes with a diameter of 28 mm and height of 150 mm filled with mineral wool and plac-

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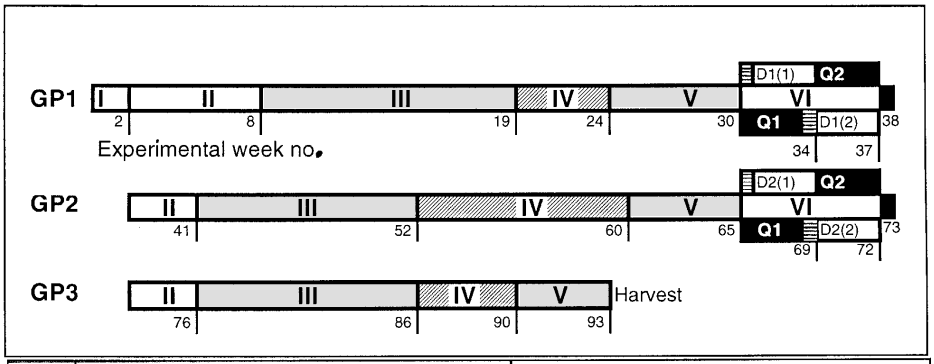
ed into racks. The spacing of the 12 plants in each rack was 7 cm x 5 cm. Five racks were placed in a plastic box fitted on a truck. Plastic ridges were placed on the bottom of the boxes to avoid exposure of the substrate to the water remaining there after watering. During the period of intensive growth of the seedlings, the boxes were filled with nutrient solution from below for 1 hour every day or every second day depending on the growth rate of the seedlings. The nutrients were provided as low concentrated complete nutrient solution of 100 mg N per litre with proportions between the main macroelements being 100N:65K:13P (INGESTAD, 1979).

3.2 Frost desiccation treatment

The temperature and light regimes during the experiment are shown in *figure 1.* After a period of hardening followed by rest breaking conditions, all the seedlings including the controls were covered by plastic bags to protect them from drying and were placed in a chamber at –3 °C in continuous darkness for one week. Afterwards, to simulate frost desiccation conditions, the seedlings with sealed tubes were placed without thawing in freezing baths filled with glycol providing –3 °C for the roots. The baths were placed in a chamber with 15 °C/5 °C day/night temperature and 12 h/12 h day/night with light intensity of 300 µmol/m²/s and 4 h at noon with high light intensity (483 µmol/m²/s) for three weeks. During each frost desiccation treatment 4 blocks with 4 plots (3 plots in one block) were used. Owing to the limited space in the glycol bath, the seedlings within each of the treatments D1 and D2 were tested at two occasions with 288 and 252 seedlings per occasion (8 and 7 seedlings per family and occasion). During the frost desiccation treatment the rest of the material including the controls was kept quiescent (see *Fig. 1*). After the frost desiccation treatment was completed all seedlings were placed in the same climatic chamber, and one week later a bud-breaking regime was started.



No.	Seasonal stage	Conditioning in the chambers		
		Temperature, Day/Night°C	Night length, h	Light intensity µmol/m²/s
I	Germination	20/20	0	300
II	Growth	20/20	0	300
		20/15	0	300
III	Growth cessation, hardening	20/15	5→8	300
		20/10	9→15	300
IV	Further hardening	20/15	16	300
		15/5	16	300
V	Rest* breaking	10/5	16	300
		5/5	16	300
		2/2	16	70
VI	**Period of frost desiccation**			
	Treatment D1, occasions 1, 2	15/5	12	300
	D1(1), D1(2), preceded by 1 week in -3°C	(roots -3)		(483 for 4 h)
	Treatment D2, occasions 1, 2	15/5	12	300
	D2(1), D2(1) , preceded by 1 week in -3°C	(roots -3)		(483 for 4 h)
	Quiescence* period 1 (Q1)	2/2	16	70
	Quiescence* period 2 (Q2)	4/4	12	70

*) The expression "rest" is used when the factor preventing growth is of physiological nature and "quiescence" is used if buds remain dormant due to low air temperature (cf. HÄNNINEN, 1990).

Fig. 1. – Temperature and light regimes during the experiment. The scheme is subdivided into growth periods (GP1 to 3) and seasonal stages of seedling development which are indicated by Roman numbers. The regimes used to induce these stages are given at the bottom of the figure. The arrows indicate successive night prolongation with 1 h per week.

3.3 Assessments

The damage caused by frost desiccation (DAMD1, DAMD2) was scored during the following growth period:

0- no observable damage;

1- apical bud on the leader dead, one of the laterals growing out;

2- dead leader, only the basal laterals flushing;

3- dead seedling.

The seedling vitality (VITALD1, VITALD2) was assessed as follows. For a dead seedling the vitality was set at 0, if the leader was not flushing the vitality was set at 0.5 and for a seedling with flushing leader the vitality was set at 1. Seedling final heights of the 1st, 2nd and 3rd growth periods were measured (HGP1, HGP2, HGP3). The height growth was also assessed at one week intervals during the 2nd and 3rd growth periods to calculate day number to reach 50% of the final leader height (DAYH50GP2, DAYH50). Assessments of phenology traits were made during the 3rd growth period. Apical bud flushing (DAYS3) on the leader was scored at 2 day intervals (dynamic period) or weekly using a scale, similar to the one developed by KRUTZSCH (1973):

0- dormant bud;

1- slightly swollen, usually a "hole" is seen if looking from above the needle bunch;

2- the bud is twice the size of a dormant bud, whitish in colour;

3- tips of the needles emerging (considered as the bud burst stage);

4- first elongation to a double of the bud length;

5- first spread of the needles at the top of the needle "brush";

6- spread of the needles at the base, the green stem is shorter than 1 cm;

7- the green stem is longer than 1 cm, the needles are spread at the base;

8- One or more tiny whitish lateral buds are visible on the stem (assumption that apical lateral bud is also set);

9- apical bud is visible.

The length of the lignified part of the leader was measured weekly at 9 occasions (DAYLIG75). At the end of the 3rd growth period all the seedlings were harvested for assessment of dry weight. The dry weight was assessed separately for the following parts of a seedling: (1) the leader (LEADDW), (2) stem of the 2nd growth period together with the needles attached to the stem (GP2STEMDW), (3) living lateral shoots grown on the stem of the 2nd growth period (GP2LATDW), (4) stem of the 1st growth period together with the laterals and the needles attached *(Fig. 2)*. The separate parts of the seedlings were placed in paper bags and dried in a drying cabinet for 64 hours in 70 °C. Number of stem units on the leader (SUN) was assessed by counting the needles during the harvest. Number of lateral buds on the leader (LEADBNO) and number of laterals, including the dead ones, grown on the stem of the 2nd growth period (GP2LATNO) were counted. Occurrence of free growth (FREE) was estimated at the harvest as the proportion of the lateral buds on the leader which had flushed from the total number of lateral buds on the leader. More weight was given to the elongating buds as follows: the lateral buds on the leader which had flushed and elongated more than 1 cm were given a value of 1, the lateral buds on the leader which had flushed or elongated less than 1 cm were given a value of 0.5. The dry weight of the stem of the 1st growth period together with the needles and laterals *(Fig. 2)* was mainly determined by biomass produced in the 3rd growth period and it was strongly correlated with total seedling dry weight (TOTALDW). Therefore, the dry weight of the stem of the 1st growth period together with the laterals was not used in the analysis, except for calculation of the total seedling dry weight. The variables derived from the traits and the transformations used are summarised in *table 1.*

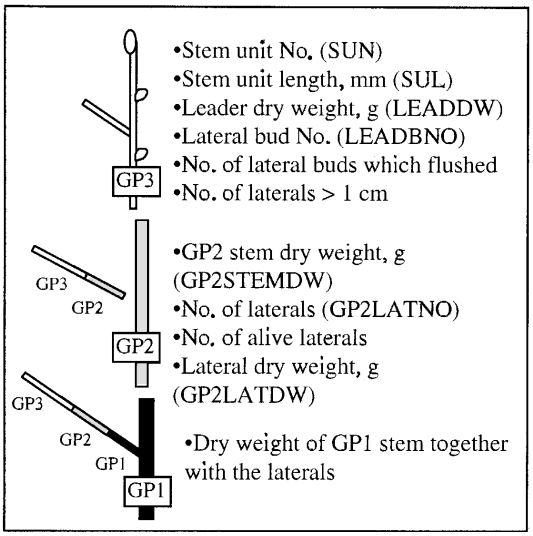


Fig. 2. – Partitioning of the seedling biomass at the harvest. The traits assessed are presented at the right side of the figure.

3.4 Statistical analyses

The ANOVA and estimation of variance components for the height growth, biomass, phenology and morphology traits *within a treatment* were performed according to the following model:

$$Y_{ijk} = \mu + B_i + F_j + \varepsilon_{ijk} \qquad [1]$$

where Y_{ijk} is an individual observation, μ is the total mean, B_i is fixed block effect, F_j is random family effect, ε_{ijk} is random error. The LEVENE's variance homogeneity test (LEVENE, 1960), W-test statistics on the model residual normality (SHAPIRO and WILK, 1965) and plot of residuals versus predicted values were used to test validity of the assumptions for ANOVA. If the assumptions were not valid a transformation was used *(Table 1).* For the damage scores (DAMD1, DAMD2) the analyses were made with the occasions pooled on family and block means and on individual level. ANOVA on the damage score in the treatment D2 showed significant difference between the two occasions and thus block effect was significant. Consequently, when making the ANOVA on the damage score on family and block means the family effect was less, owing to the block effect. To illustrate this the results are presented both from family block mean and individual levels.

The ANOVA and estimation of variance components for the height growth, biomass, phenology and morphology traits *with all the treatments pooled* were performed on individual level by the following model:

$$Y_{ijk} = \mu + T_i + F_j + (TF)_{ij} + \varepsilon_{ijk} \qquad [2]$$

where Y_{ijk} is an individual observation, μ is the total mean, T_i is fixed treatment effect, F_j is random family effect, $(TF)_{ij}$ is random effect of interaction between treatment and family, ε_{ijk} is random error. As the block effect was not significant it was excluded from the model. The assumptions for ANOVA were

Table 1. – Description of the variables and the transformations used in the ANOVA. For the variables which were not transformed in the ANOVA, the transformation is indicated as "none". For seedling vitality after the treatments (VITALD1, VITALD2) which were not used in the ANOVA, the transformation is indicated as "–". In the transformation column arc sine is abbreviated as "arcsin", square root is abbreviated as "sqrt".

Variable	Description	Transformation
	Frost desiccation damage	
DAMD1	Damage score in the treatment D1 at the later part of the rest period after the 1st growth period (GP)	arcsin(sqrt(p/3))
VITALD1	Seedling vitality after the treatment D1	–
DAMD2	Damage score in the treatment D2 at the later part of the rest period after the 2nd GP	arcsin(sqrt(p/3))
VITALD2	Seedling vitality after the treatment D2	–
	Height growth traits	
HGP1	Final height in GP1, mm.	Log(p)
HGP2	Final height in GP2, mm	Log(p)
HGP3	Final height in GP3, mm	Log(p)
LEADHGP2	Final leader height in GP2, mm	none
LEADH	Final leader height in GP3, mm	Log(p+10)
SUN	Stem unit number on the leader in GP3	Log(p)
SUL	Stem unit length of the leader in GP3, mm	none
	Phenology and morphology traits	
GP2LATNO	Total number (alive and dead) of laterals on GP2 stem	none
LEADBNO	Number of lateral buds on the leader of GP3	none
FREE	Estimate of free growth in GP3 (see Material and methods)	Log(p+0.5)
DAYH50GP2	Day No. since the end of the treatment D1 to reach 50 % of the final leader height in GP2	Log(p+1)
DAYH50	Day No. since the end of the treatment D2 to reach 50 % of the final leader height in GP3	Log(p+5)
DAYLIG75	Day No. since the end of the treatment D2 to lignify 75% of the final leader height in GP3	Log(p+5)
DAYS3	Day No. since the end of the treatment D2 to reach flushing stage 3 in GP3	Log(p+5)
	Biomass traits	
LEADDW	Leader dry weight in GP3, g	Log(p)
GP2STEMDW	Dry weight of the stem of GP2 together with the needles attached to the stem, g	Log(p+1)
GP2LATDW	Dry weight of the alive laterals grown on GP2 stem, g	Log(p+1)
TOTALDW	Total seedling dry weight at the end of GP3, g	Log(p+5)

tested as described above. Seedlings on which apical buds did not flush, i.e. were dead or had dead leader, or which were classified as suppressed by competition, were excluded from all the analyses.

The type "A" genetic correlations among the variables measured on the same individual within a treatment were calculated using the sum of two variables. Before calculation of the variance component for the sum of two variables, these variables were standardised to have zero means and unit standard deviation. The genetic correlation coefficient was calculated by the following formula:

$$r_{gA} = \frac{4\sigma_{f(xy)}}{\sqrt{4\sigma^2_{f(x)} 4\sigma^2_{f(y)}}} \qquad [3]$$

where $\sigma_{f(xy)}$ is genetic covariance between two variables, and $\sigma^2_{f(x)}$ and $\sigma^2_{f(y)}$ are family variance components. Standard error for the genetic correlation was estimated according to FALCONER (1989).

The genetic correlations of type "B" (BURDON, 1977) between variables measured in different treatments were calculated according to the following formula:

$$r_{gB} = \frac{r_{xy}}{\sqrt{h^2_{f(x)} h^2_{f(y)}}} \qquad [4]$$

where r_{xy} is product-moment correlation among the family means, and $h^2_{f(x)}$ and $h^2_{f(y)}$ are family heritabilities, which for half-sibs were estimated as follows (e.g. FINS et al., 1992):

$$h^2_f = \sigma^2_f / (\sigma^2_f + \sigma^2_e / k) \qquad [5]$$

where k is harmonic mean of number of observations per family and treatment.

To illustrate significance of the type "B" genetic correlation, the significance value of the product-moment correlation among family means is presented (cf. BURDON, 1977).

The analyses were performed using the CORR, GLM and MIXED procedures in the SAS statistical package for personal computers (SAS Institute, 1987).

4. Results

4.1 External damage caused by frost desiccation

Both the frost desiccation treatments, D1 and D2, had significant effect on the subsequent seedling development. As a first sign of the damage, marked discoloration of the needles on the treated seedlings was observed during the bud flushing three weeks after the treatment. Besides reduced height growth, the visually observable damage varied from dead leader to completely dead seedling in the succeeding growth period. No significant abnormalities in growth of the control seedlings were observed. The D2 seedlings had significantly lower vitality

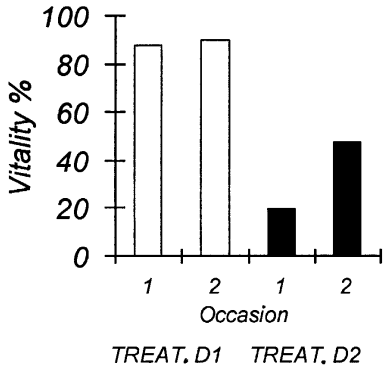


Fig. 3. – Seedling vitality after the frost desiccation treatments after the 1st (TREAT. D1) and 2nd (TREAT. D2) growth periods. The mean values are presented for each occasion of the frost desiccation treatments.

than the D1 seedlings and the D2 seedlings tested at the 1st occasion had significantly lower vitality than the D2 seedlings tested at the 2nd occasion *(Fig. 3)*. The family effect on the damage score was significant in both the frost desiccation treatments *(Table 2)*. The block effect was significant in D2 only. This significance was caused by difference in the damage between the occasions. The type "B" genetic correlation between the damage score in treatment D1 and D2 was significant (r_{gB}= 0.93, the product-moment correlation among the family means r_p= 0.33*).

4.2 Height growth traits

The ANOVA on height of the seedlings without external damage (the damage score was 0) showed that the seedlings affected by frost desiccation grew significantly less than the controls *(Table 3, Fig. 4)*. Thus, in the 2nd growth period, the D1 seedlings were significantly shorter than the control seedlings. Similarly, in the 3rd growth period, the D2 seedlings had significantly shorter leaders than the controls, while the D1 seedlings had reached the height of the D2 seedlings *(Table 3, Figs. 4, 5)*. During the 3rd growth period the D1 seedlings had significantly fewer but significantly longer stem units than the controls *(Table 3, Fig. 6)*.

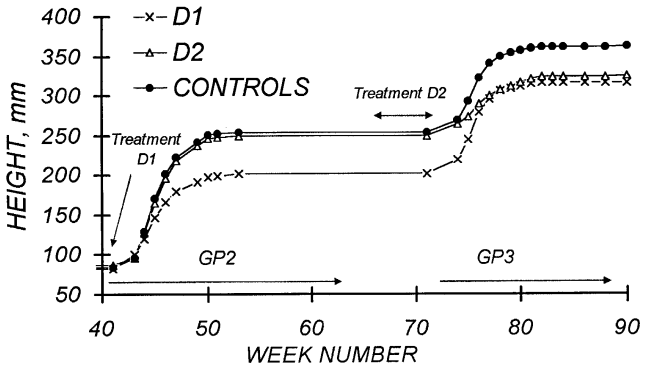


Fig. 4. – Height growth of the seedlings treated after the 1st (D1) and 2nd (D2) growth periods in comparison with the controls (C). Experimental week number from the start of the experiment (seeding) is given on the X-axis. The frost desiccation treatments are indicated by the arrows.

The family effect was significant for all height growth traits. The highest family variance components were obtained for final heights of the seedlings, and the lowest for the leader heights in the 2nd and 3rd growth periods *(Table 3)*. The variance component for family x treatment interaction was lower than the family component in all cases except for leader height in the 3rd growth period *(Table 3,* see also *Fig. 5)*. The correlation coefficients between the treatments indicated that the interactions for the traits of the 3rd growth period were caused by the treatment D2 (not presented). The family variance components for final height of the 1st and 2nd growth period were of the same magnitude independently of the treatment *(Table 4)*. However, the family variance components for the final heights in all treatments roughly halved from the 1st to the 2nd growth period. D2 had the strongest family control of

Table 2. – Results from the ANOVA on the damage score after the treatment D1 simulating frost desiccation after the 1st growth period (DAMD1) and after the treatment D2 performed after the 2nd growth period (DAMD2). Significance levels of the F statistics are presented. The damage scores were arc sine square root transformed. "F/P means" show results from the analyses on family and plot means.

Source	DAMD1				DAMD2			
	Individual level		F/P means		Individual level		F/P means	
	DF	P>F	DF	P>F	DF	P>F	DF	P>F
BLOCK	3	0.1498	–	–	3	0.0001	–	–
FAMILY	35	0.0269	35	0.0301	35	0.0014	35	0.1433
ERROR	501		108		498		108	
TOTAL	539		143		536		143	

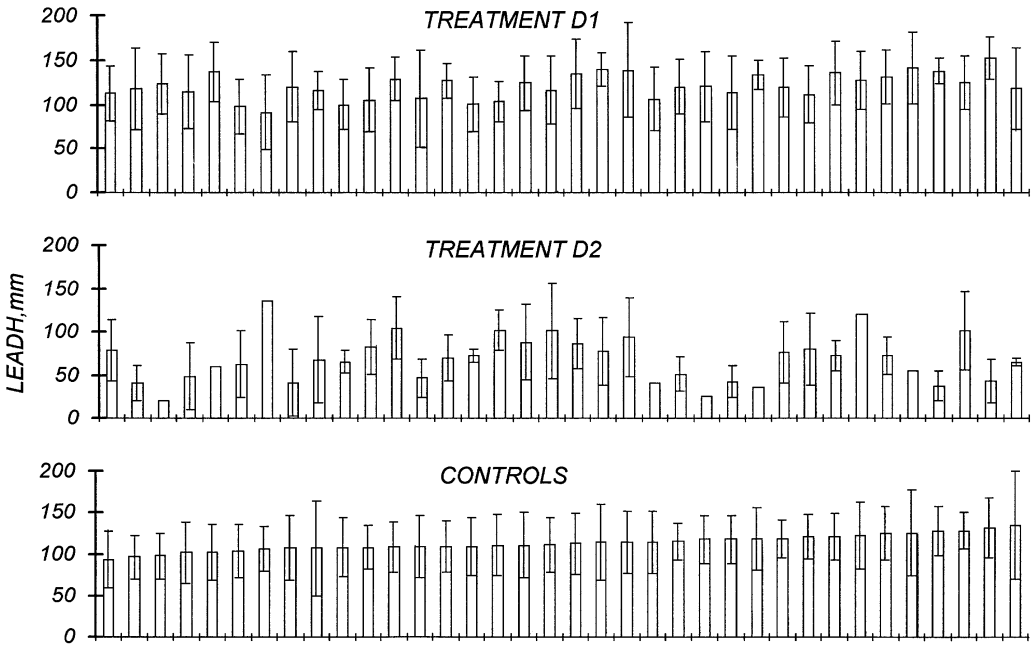


Fig. 5. – Family mean height of the leader (LEADH) in the 3rd growth period within each of the treatments. The families in the treatments D1 and D2 are ordered according to the ranking of the control families. The error bars show standard deviation. The standard deviation bars are absent for some of the family mean values in the treatment D2, because there was only 1 seedling per family available.

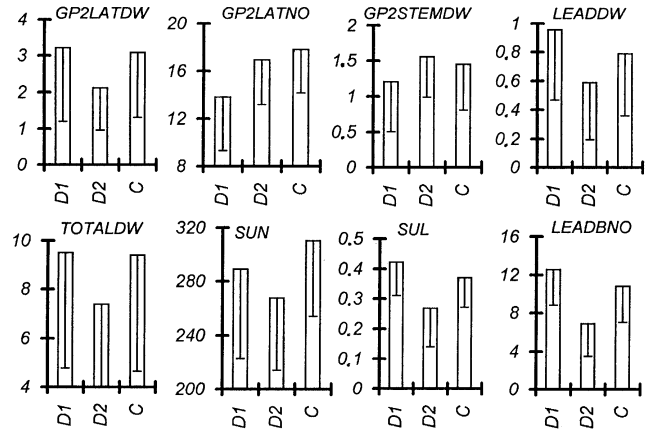


Fig. 6. – Treatment mean values and standard deviations for the height growth, biomass and morphology traits. D1, D2 are the frost desiccation treatments and C is the control.
GP2LATDW = dry weight in grams of alive laterals on GP2 stem; GP2LATNO = total No. of laterals on GP2 stem; GP2STEMDW = dry weight in grams of the stem of GP2 together with the needles attached to the stem; LEADDW = leader dry weight in grams in GP3; TOTALDW = total seedling dry weight in grams at the end of GP3; SUN = stem unit No. on the leader of GP3; SUL = stem unit length of the leader in millimetres in GP3; LEADBNO = number of lateral buds on the leader of GP3.

Table 3. – Results of the ANOVA with all the treatments pooled. Variance components and standard errors for the random effects are presented as percentage from the total variation. For the treatment effect significance of the F statistics is given (p). The treatments are compared in the last column, where: the treatments significantly different at 0.05 level according to the TUKEY test are indicated by "<" or ">". Abbreviations of the variables are explained in *table 1.*

Variable	Random effects				Fixed treatment effect	
	FAM	S.E.	F*T	S.E.	p	Comparison
			Height growth traits			
HGP2	14.5	5.5	4.5	2.5	0.0001	(C=D2)>D1
HGP3	10.1	3.6	1.0	2.1	0.0001	C>(D1=D2)
LEADHGP2	3.5	1.5	0.4	1.4	0.0001	(C=D2)>D1
LEADH	2.9	2.3	6.7	3.6	0.0001	D1>C>D2
SUN	7.5	3.0	2.1	2.2	0.0001	C>D1>D2
SUL	5.6	2.6	3.8	2.7	0.0001	D1>C>D2
			Phenology and morphology traits			
GP2LATNO	7.2	2.5	2.1	1.6	0.0001	(C=D2)>D1
LEADBNO	0.0	-	1.8	1.9	0.0001	D1>C>D2
FREE	3.9	2.2	1.1	2.3	0.0042	(D1=C)>D2
DAYS3	7.8	3.3	6.3	3.4	0.0015	(D2=C)>D1
			Biomass traits			
GP2STEMDW	4.0	2.1	1.1	2.0	0.0001	(C=D2)>D1
GP2LATDW	8.0	3.1	2.1	2.1	0.0001	(D1=C)>D2
LEADDW	3.0	1.9	0.9	2.3	0.0001	D1>C>D2
TOTALDW	12.8	4.3	3.0	2.4	0.0320	(D1=C)>D2

the leader height in the 3rd growth period *(Table 4, Fig. 5)*, but the weakest control of the final seedling height of the 3rd growth period *(Table 4)*.

Table 4. – Family variance components and standard errors expressed as percentage of the total variation. The variance components were calculated separately within each treatment. Harmonic means (HRM) of number of the observations per family and treatment are given separately for each of the treatments. Abbreviations of the variables are explained in *table 1.*

Variable	Treatment D1			Treatment D2			Controls		
	HRM	FAM	S.E.	HRM	FAM	S.E.	HRM	FAM	S.E.
				Height growth traits					
HGP1	15.0	39.3	10.2	15.0	31.6	8.4	15.0	37.7	9.5
HGP2	11.0	18.2	6.2	14.0	18.0	5.8	13.8	19.4	6.1
HGP3	9.2	13.1	5.6	3.6	3.2	7.4	11.6	10.6	4.6
LEADHGP2	11.0	6.8	3.6	13.9	3.1	2.4	13.8	1.5	2.1
LEADH	9.2	7.4	4.3	3.7	18.6	9.3	11.6	0.0	-
SUN	9.2	10.0	4.9	3.7	9.0	8.9	11.5	9.3	4.3
SUL	9.2	9.8	4.6	3.7	21.9	10.4	11.5	4.3	3.1
				Phenology and morphology traits					
GP2LATNO	10.7	7.8	3.9	14.0	8.0	3.5	13.1	11.8	4.5
LEADBNO	9.2	3.1	3.4	3.7	7.9	7.3	11.6	0.4	2.1
DAYH50GP2	10.8	9.5	4.3	13.9	8.0	3.5	13.8	9.0	3.8
DAYH50	9.1	4.7	3.7	3.4	1.7	7.0	11.6	4.2	3.0
FREE	9.2	4.0	3.6	3.7	15.4	9.5	11.6	3.5	2.9
DAYS3	9.2	2.2	3.0	3.7	5.5	8.2	11.6	21.9	6.9
DAYLIG75	9.1	1.8	3.1	3.4	0.0	-	11.6	6.2	3.4
				Biomass traits					
GP2STEMDW	10.4	3.8	3.2	4.2	3.8	6.4	13.1	7.5	3.7
GP2LATDW	10.1	9.8	4.5	4.2	10.0	7.3	12.9	11.9	4.6
LEADDW	9.2	3.2	3.4	3.6	13.3	8.1	11.6	1.5	2.5
TOTALDW	9.1	13.7	5.6	3.6	27.3	13.4	11.5	14.4	5.4

4.3 Biomass traits

In comparison with the controls the seedlings exposed to the frost desiccation produced less biomass *(Table 3, Fig. 6)*. The control and D2 seedlings had significantly higher dry weight of the stem with the needles of the 2nd growth period than the D1 seedlings. Leader dry weight of the D2 seedlings was significantly lower than that of the control and D1 seedlings, which had the heaviest leaders in the 3rd growth period. During the 3rd growth period the D1 seedlings had significantly fewer but heavier laterals on the stem of the 2nd growth period than the control seedlings. The D2 seedlings had the lowest total seedling dry weight. Family effect on the biomass traits, except leader dry weight, was equally important in all treatments *(Table 4)*. The D2 seedlings showed stronger family control of leader dry weight and total seedling dry weight than the D1 and control seedlings. Family x treatment interaction in the biomass traits was not significant *(Table 3)*.

4.4 Phenology and morphology traits

In comparison with the controls the D1 seedlings produced significantly fewer lateral shoots during the 2nd growth period, while the D2 seedlings had significantly fewer buds on the leader of the 3rd growth period *(Table 3, Fig. 6)*. After the treatment the D2 seedlings flushed slightly later and exhibited less free growth than the control and D1 seedlings, which had the earliest flushing *(Table 3)*. The family effect was significant for all the phenology and morphology traits except for the number of lateral buds on the leader *(Table 3)*. Family x treatment interaction in phenology and morphology traits was strongest for bud flushing. The controls were distinguished by high family control of bud flushing *(Table 4)*.

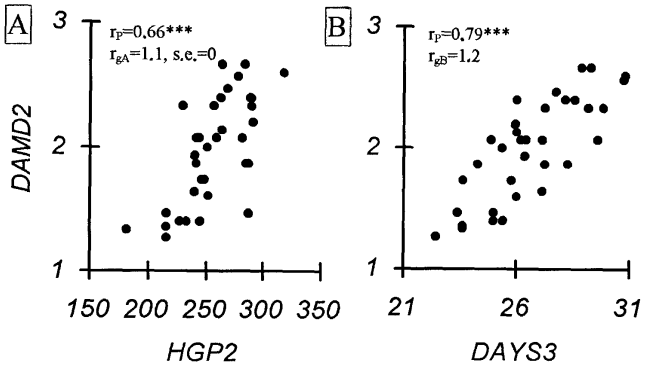


Fig. 7. – Plot of family means of the damage score (DAMD2) in the treatment D2 and (A) final height of the 2nd growth period for the treatment D2 seedlings (HGP2), (B) days to reach bud flushing stage 3 for the controls (DAYS3). Genetic (r_{gA} – of type "A" and r_{gB} – of type "B") and PEARSON (r_p) correlation coefficients are presented in the upper left corner of a corresponding plot.

Table 5. – Correlations between the damage score in treatment D2 and height growth traits as well as phenology traits of treatment D2 and of the controls. The traits of the controls are indicated by "C", the traits of the treated seedlings by "D2". Standard errors of genetic correlations among the traits within treatment D2 (type "A") are given below the coefficient. The genetic correlations of type "B" were calculated between the damage score and traits in the controls.

Trait	DAMD2	
	Genetic correlations	Pearson correlations
$HGP1^{D2}$	1.06	0.75
	0.00	0.0001
$DAYH50GP2^{D2}$	0.70	0.44
	0.23	0.0070
$HGP3^{C}$	0.72	0.38
		0.0240
$DAYLIG75^{C}$	1.19	0.57
		0.0003
$DAYH50^{C}$	0.94	0.39
		0.0177

4.5 Relationship between the damage score and height growth and phenology traits

The genetic variation in the damage score assessed in the 3rd growth period on the seedlings in the treatment D2 was significantly positively correlated with the genetic variation in the height growth and phenology traits assessed on the same seedlings in the 1st and 2nd growth periods *(Table 5, Fig. 7A)*. The genetic correlations between phenology traits of the controls and the damage score in the treatment D2 were strong. The late flushing families were more damaged after frost desiccation treatment than early flushing families *(Fig. 7B)*.

5. Discussion

5.1 General

Exposure to a relatively high temperature (+15 °C/5 °C day/night) and to 4 hours of intensive light at noon during a period of 3 weeks was the only significant difference in treat-

ment between the frost desiccation treated seedlings and the controls. It is unlikely that the observed effects after the treatments could be attributed to low root hardiness, since there was no visible damage on roots. Moreover, the growth conditions were designed to bring about plant hardiness. A few weeks after the treatment the needle colour of the damaged seedlings changed to dull green but not to reddish brown which is considered as a symptom of winter frost damage as discussed by STRIMBECK et al. (1991).

Larger seedlings having greater transpiring needle surface may lose water with a higher rate than short seedlings. This may have caused more damage in D2 than in D1. We wanted to maintain similar hardiness level of the seedlings tested at both occasions within the treatments D1 and D2, respectively. While the occasion 1 seedlings were treated, the occasion 2 seedlings were kept quiescent for 3 weeks. During these 3 additional weeks at 2°C we may have increased hardiness of the seedlings. Increase of hardiness in *Picea abies* is associated with closure of stomata and decrease in transpiration rate (CHRISTERSSON, 1972). Thus, the D2 seedlings treated at the 2nd occasion would be more hardy with less damage than the seedlings treated at the 1st occasion.

The low vitality in the D2 treatment seriously influenced the possibility of revealing the genetic variation in this treatment. Thus, only 3 traits assessed during the 3rd growth period had a standard error of the family variance component less than 50 % of the estimate for the trait itself *(Table 4).*

5.2 The family variation in external damage caused by frost desiccation

More than half of the treated seedlings had multiple leaders or no clear leader at all. Repeated exposure to frost desiccation would lead to a bushy crown known as "krummholz" resulting from frost desiccation at high altitudes (TRANQUILLINI, 1976). Family effect for the damage score was significant, even though the total variation in the damage in D1 and D2 was relatively low *(Fig. 3).* The genetic correlation between the damage score in D1 and D2 was strong which strengthens the reliability of our findings. Owing to the common ancestry in the continental Europe and further growth in similar environments in southern Sweden, the families in our study behaved more like representatives of one population rather than of populations of genotypically contrasting origins. If the stabilising selection was strong in the previous generations, the within-population variation would be low unless this was counteracted by a strong gene flow. Support for the action of strong stabilising selection was given by HATAKEYAMA (1981) who showed that variation in frost desiccation damage among *Abies sachalinensis* half-sib families was much less than that between provenances.

5.3 Frost desiccation effect on the family variation in height growth and biomass traits

From *table 4* it is evident that the family variance component for height growth was extremely high during the 1st growth period in all the treatments. With the assumption that half-sib families estimate 1/4 of the additive genetic variance, the heritabilities would far exceed 1.0. Although there was no significant relationship between the family seed weights and family heights in our study it is assumed that a part of the family effect must be attributed to maternal effects (cf. MIKOLA, 1980).

In spite of the reduced height growth caused by the D1 treatment, the family variance components for HGP2 were rather similar in all the three treatments *(Table 4).* Large family differences in SUL and total seedling biomass in the D2 treatment are evident, whereas there was a limited variation in SUL among the families in the control material. The D1 treatment caused a larger family differentiation in the leader height of the 2nd growth period (6.8 ± 3.6) than in the two untreated materials (3.1 ± 2.4 and 1.5 ± 2.1) at this stage of the experiment. Similarly, the D2 seedlings exhibited a stronger family control of leader height in the 3rd growth period than the untreated control seedlings *(Table 4, Fig. 5).*

The absence of family variation in leader height and weight at the 3rd growth period in the control material is surprising. The control seedlings were larger and had to face a stronger competition than the seedlings in all the other treatments in this single-tree-plot experiment. According to FRANKLIN (1979) this would lead to a stronger differentiation among the genetic entries. Thus, a more severe competition in the control material than in the two other treatments can probably be ruled out as a cause for the absence of family variation in this control material.

The interaction variance components were always less than the family variance components, except for leader height of the 3rd growth period *(Table 3).* This interaction was mainly caused by the absence of the family variation for this trait in the control material and is thus purely mathematical *(Table 4).* The limited importance of G x E interaction in the presence of the treatment effects on the traits suggests that phenotypic plasticity is of adaptive significance. This is expected for a long-living and wind pollinated species that grows over a wide span of site conditions (ERIKSSON, 1997).

The seedlings affected by frost desiccation grew significantly less in the following growth period and, thus, produced less biomass than the controls because they had fewer and shorter stem units than the controls *(Figs. 4, 6).* Desiccation can restrict length of stem units in conifers (GARRETT and ZAHNER, 1973). The number of stem units initiated in the buds formed during the growth period before the treatment is expected to be similar in the treatment and control seedlings. However, owing to stress a part of the stem units in the buds of the treated seedlings may have aborted (GARRETT and ZAHNER, 1973) which would explain the smaller stem unit number in D2 than in the controls. The buds initiated one growth period after the treatment were affected by the desiccation as shown by the fewer stem units in the buds of D1 seedlings than in the controls *(Fig. 6).* During the 3rd growth period D1 seedlings in response to the stress had elongated the stem units more than the controls and, in spite of fewer stem units, had equally long and heavier leaders in comparison with the controls.

The reduced height growth of the treated seedlings means that frost desiccation may affect the seedlings without any externally observable damage. Under natural stresses, the majority of such frost-desiccated but seemingly undamaged seedlings may be eliminated by competition.

5.4 Frost desiccation effect on phenology and morphology traits

Presumably, frost desiccation disturbed normal metabolism of the seedlings which had to repair the damage. This may have caused the differences in phenology and morphology traits between the treatment and controls. We observed that the D2 seedlings had a slightly later and the D1 seedlings had significantly earlier bud flushing than the control seedlings in the 3rd growth period. This may have weakened family control and strengthened family x treatment interaction in timing of bud flushing *(Tables 3, 4).* Reduced vitality of the treated seedlings prevented formation of a large number of lateral buds and occurrence of free growth *(Table 3, Fig. 6)* which is in agreement with WENGER's (1952) study on *Pinus* seedlings.

5.5 Relationship between tolerance to frost desiccation and frost hardiness

Families with short, early flushing seedlings with early lignification of the leaders were more tolerant to frost desiccation than families with tall and late flushing seedlings *(Table 5, Fig. 7).* Our findings are in agreement with a report by DORMLING (1993) on higher tolerance to frost desiccation in a Norway spruce population from southern Sweden than in a population from Belarus, which had taller and less frost hardy, later flushing seedlings than the Swedish population. A corresponding pattern was observed by HANNERZ (1994): *Picea abies* grafts which ceased growth early tended to be less damaged by frost desiccation than the grafts ceasing growth late in a clonal seed orchard located in central Sweden. The studies on tolerance to frost desiccation of other conifers also support our results. Populations with late growth cessation are more vulnerable to frost desiccation in late winter than populations with early growth cessation (DIETRICHSON, 1969; STÅHL and PERSSON, 1992 for *Picea mariana;* BONGARTEN and HANOVER, 1986 for *Picea pungens)*. Frost desiccation damage increased with increasing height of *Picea glauca* seedlings (KRASOWSKI et al., 1996) and *Cryptomeria* plants (MURAI and FURUKOSHI, 1976). LARSEN (1978) found that *Pseudotsuga menziesii* originating from high altitudes was frost desiccated more slowly than the origins from the lower latitudes. HATAKEYAMA (1981) reported less damage by frost desiccation on *Abies sachalinensis* provenances originating from the areas where severe cold and desiccation occurs during winter. EIGA (1984 cited in SAKAI and LARCHER, 1987) found positive correlation between resistance to frost desiccation and early flushing in *Abies sachalinensis* provenances. CHRISTERSON et al. (1987) reported that *Picea abies* was more tolerant to frost desiccation than *Picea mariana.* They concluded that *Picea mariana* is adapted to more humid climates than *Picea abies.* Frost desiccation usually damages plants after the severe winters frequent at higher latitudes and altitudes (SAKAI and LARCHER, 1987). Thus, populations from such climates are expected to be better adapted to withstand the stresses induced by cold winters than populations from milder climates. Southerly populations of *Picea abies* show later bud flushing and budset, grow taller and possess less winter frost hardiness than northerly populations (DORMLING, 1979, 1993; KRUTZSCH, 1986; PERSSON and PERSSON, 1992). The same pattern of relationships among the traits exists within populations, as experienced by us here and e.g. EKBERG et al. (1985). Frost hardiness may be an important factor in tolerance to frost desiccation. Presumably, seedlings which terminate growth late may not be able to attain a sufficient degree of frost hardiness before occurrence of frosts in autumn which may disturb the development of hardiness. For instance, cold limits maturation of needles in *Picea abies* seedlings if low temperatures occur during maturation (BAIG et al., 1974) or causes inadequate shoot periderm formation in *Picea rubens* shoots if the formation occurs too late in the season (EVANS and BIESEMEYER, 1988). Reduced hardiness of *Picea rubens* provenances led to winter injury (DEHEYES et al., 1990). TRANQUILLINI (1979) reported that frost during shoot growth is responsible for disturbances which lead to subsequent winter frost desiccation of conifers at the timberline in the Alps. If conifer seedling shoots are unable to reach full maturity in autumn they may transpire too strongly during the winter (HOLTMEIER, 1971). All these factors may increase susceptibility to frost desiccation. QAMARUDDIN et al. (1993) and DORMLING (1993) demonstrated that frost hardiness in *Picea abies* seedlings increases while dormancy is lost. Consequently, seedlings from northerly populations though flushing early possess a higher degree of hardiness and, thus, tolerance to desiccation than seedlings of southern populations. In our study this is supported by strong genetic correlations between the damage score and seedling height as well as the phenology traits. This indicates that the tolerance to frost desiccation is to a large extent regulated by the same or linked genes as involved in the regulation of seedling height and phenology traits. Genetic control of phenology traits in *Picea abies* is strong and of additive nature (EKBERG et al., 1982, 1985, 1991). This would make breeding for tolerance to frost desiccation efficient, assuming involvement of the same genes as in phenology traits. Prior selection of provenance is important.

In conclusion, our experiment shows that (1) in seedlings of *Picea abies,* simulated frost desiccation resulted in decreased height growth, defects or death (2) family effect for the growth traits was more important than the family x treatment interaction (3) family effect in tolerance to frost desiccation was significant and (4) early flushing and short seedlings survived the desiccation treatment better than late flushing and tall seedlings. This tolerance must be attributed to the high degree of hardiness possessed by early flushing and short seedlings.

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Systematics and Genetic Structure of Washoe Pine: Applications in Conservation Genetics

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Abstract

Independent studies of seedling populations of Washoe *(Pinus washoensis)* and ponderosa *(P. ponderosa)* pines grown in common gardens demonstrated that: (1) the systematic relationship between Washoe pine and the North Plateau race of ponderosa pine is close and (2) the allocation of genetic variability among and within populations of the narrow endemic, Washoe pine, is similar to that of the broadly dispersed ponderosa pine. The results from this quantitative analysis of adaptive traits thus support previous works involving morphology, terpene chemistry, allozyme variation, mating systems, DNA biochemistry, and classical taxonomy that lead to a conclusion of synonymity for Washoe pine and ponderosa pine. The results also provide no genetic evidence that small population sizes and isolated distributions have had deleterious genetic consequences. Populations of Washoe pine nevertheless have unique characteristics that may be worthy of conservation. Programs should concentrate on habitat preservation and range expansion.

Key words: Pinus washoensis, quantitative traits, genetic structure, systematics, conservation biology.

Introduction

Loss of habitat, restricted distributions, isolation, and inbreeding lead toward the deterioration of genetic variability, loss of genes, extinction of populations, and, ultimately, endangerment of species. Estimating the risk before endangerment challenges conservationists to accumulate and synthesize disparate sources of information. Besides demographics, phylogenetic and systematic relationships must be considered together with the factors molding the system of genetic variability (AVISE,

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