FOREST GENETICS 3(2):69–79, 1996

DIALLEL CROSSES IN *PICEA ABIES*. II. PERFORMANCE AND INBREEDING DEPRESSION OF SELFED FAMILIES

Tore Skrøppa

Norwegian Forest Research Institute , N–1432 Ås, Norway

Received April 30, 1996; accepted July 29, 1996

ABSTRACT

Complete 10 × 10 diallels were performed within each of three natural Norway spruce populations. The performance of selfed and outcrossed families was compared for a large number of traits measured in a nursery test and in a short term field trial kept until the trees were 10 years old. The mean performance of the selfed families was significantly different from the mean of the outcrossed families for almost all traits studied. They germinated more slowly, had an earlier bud set, a shorter and differently timed shoot growth period with a lower rate of growth, and a reduced height and diameter growth. The mean inbreeding depression was significantly different from zero for most traits, in spite of the fact that only additive genetic variance was found in the statistical analyses of outcrossed families. A large variation in inbreeding depression was found among the selfed families. Different correlation patterns were observed in the three populations, both for the same trait measured on selfed and outcrossed families, and for inbreeding depression estimates of different traits. The results are discussed and interpreted based on the present knowledge of the causes of inbreeding depression and its variability.

Key words: *Picea abies*, self fertilization, inbreeding depression, germination, mortality, height growth, growth rhythm

INTRODUCTION

In most forest tree species, inbreeding strongly affects the performance and variation of most traits. This fact is of significant importance both from the evolutionary point of view and for practical breeding. Studies of inbreeding have focused both fitness traits such as seed abortion and germination rates, see reviews by FRANKLIN (1969) and KÄRKKÄINEN (1994), and growth and vigour characters (*e.g.* WILCOX 1983; HARDNER & POTTS 1995; MATHESON *et al.* 1995; DUREL *et al.* 1996). The highest level of inbreeding occurs after self-pollination, and a majority of the reported results relate to the inbreeding depression (reduction in fitness, vigour and growth) occurring in selfed families. In conifers, selfing generally lowers the seed yield and reduces plant survival and height and diameter growth (FRANKLIN 1969). There is, however, a large variation in inbreeding depression both among species (FRANKLIN 1969), among populations of the same species and among trees of the same population (KÄRKÄINEN *et al.* 1996). A review of inbreeding depression in conifers and its application in breeding was recently published by WILLIAMS and SAVOLAINEN (1996).

In Norway spruce (*Picea abies* (L.) Karst.), controlled self-pollinations were successfully attempted as early as in 1909 (SYLVÉN 1910), and an experimental plantation with 4 selfed families and open pollinated progenies from the same mother trees was established. The selfed progenies in this plantation have suffered both a higher mortality and a reduced height and diameter growth compared with their outcrossed progenies (LANGLET 1941; ERIKSSON *et al.* 1973). New crosses involving different levels of inbreeding were made in the plantation (ANDERSSON 1965; ANDERSSON *et al.* 1974), and results from subsequent experiments confirmed the inbreeding depression in filled seed yield and early height growth. However, the observed inbreeding depression for height growth varied among families and was also affected by the environmental conditions.

Complete diallels were performed within three natural Norway spruce populations as part of the genetic architecture studies in the species. Results concerning seed yield and seed weight have previously been reported by SKRØPPA & THO (1990). This article presents results from studies of the performance and variation of the selfed families in nursery and in a short term field test kept until the trees were 10 years old. The selfed progenies were compared with the outcrossed families from the same parents, and variability patterns in inbreeding depression were studied both within and between populations. The reported traits characterized germination, mortality, tree form, height and diameter growth and growth rhythm. The results are discussed relative to the present knowledge of the mechanisms generating inbreeding depression and its variability.

MATERIALS AND METHODS

Materials

A complete diallel cross, including both reciprocal crosses and self-pollinations, was performed in 1973 between 10 trees within each of three natural stands of Norway spruce. The parents were random trees, standing more than 50 meters apart, that had both female and male flowers. The three stands were all located in the same region in south-eastern Norway and were less than 60 km apart. Their respective altitudes were 500 m (Diallel 1, Veldre), 270 m (Diallel 2, Gjøvik) and 300 m (Diallel 3, Braskereidfoss). More details of the stands and the crossing procedures are given by SKRØPPA & THO (1990).

Due to non-functioning pollen from one of the parents in Diallel 3, the dimension of this diallel had to be reduced to 9 x 9. Filled seeds were obtained for most of the other crosses.

Experiments

Filled seeds were sown and germinated in multipot containers in the spring of 1974 at the experimental farm Hoxmark, Ås. The germination and initial growth took place in an unheated greenhouse for the first two months until the containers were placed outside on June 18. The seedlings were grown in the containers for two growing seasons, each of the three populations grown as a separate experiment in a randomized design with six replicates of family plots of 31–32 sown seeds.

In the spring of 1976, after two growing seasons, all families with a sufficient number of seedlings were planted in a trial in an agricultural field at the experimental farm Hoxmark. Each population diallel was considered as a separate experiment, but the three diallels were grown adjacent to one another in the same field. The design was 12 randomized complete blocks (replicates) of 4-tree family plots at a spacing of 0.6 m. Of the two types of families, selfed (SELF) and outcrossed (OC), the following with less than 48 viable seedlings were excluded: 1 SELF family from Diallel 1, 5 OC and 2 SELF families from Diallel 2 and 1 OC and 5 SELF families from Diallel 3. The whole trial therefore contained 21 SELF and 246 OC families.

The field trial was kept for 8 growing seasons until the trees were 10 years old from germination. After 5 seasons, systematic thinnings were made in the trial, leaving 4 replicates of each diallel unthinned, 4 replicates thinned to 2 trees per plot and 4 replicates thinned to only 1 tree per plot.

Measurements

In the nursery experiment, germination was assessed after 10 days, and a final germination record was made after 35 days. The germination rate was calculated as the percentage of germinating seeds that had germinated after 10 days, and the germination percentage was based on the total number of seeds sown. The heights of 15 seedlings of each plot was measured to the nearest 0.5 cm at the end of the first and second growth season. At the end of the second growth season, the number of plants that had set terminal bud was counted in each plot of 3 replicates, and the percentage of seedlings per plot with terminal bud was calculated.

In the field trial, heights of all trees were measured at a total age of 5, 7 and 10 years. The accumulated height increments of growing seasons 6 and 7 and 8, 9 and 10, respectively, were calculated by subtraction. The diameter at 0.5 m tree height was measured at age 10. When the experiment was terminated at age 10, stem discs were collected at 0.5 m tree height, and the basic wood density was determined by the water deplacement method (OLESEN 1971).

At the end of the seventh growing season, each tree was assessed for its form, whether it was normal (treelike) or non-normal (dwarf, bushy, snake tree). After an attack of pineapple gall aphids (*Adelges spp.*) at age 7, each tree was assessed as being attacked or not. In the sixth and seventh growth season when considerable lammas growth occurred, the occurrence of a second flush was recorded for each tree. In the same years, the number of branches in that year's whorl was counted.

During the sixth (1979) and seventh (1980) growing seasons measurements of the elongation of the terminal shoot were made weekly, on all trees in 6 replicates that had a normal shoot development. The elongation after a second flush was not included. Weibull distribution functions were fitted to the relative shoot elongation measurements of each tree for each of the two years by the method described by SKRØPPA and MAGNUSSEN (1993). Based on the estimated Weibull curves the following shoot elongation characteristics were calculated: the day of growth start, the day of growth cessation, the duration of the growth period and the rate of shoot elongation.

Calculations and statistical analyses

For each parent, the mean of its outcrossed offspring (OC family mean) was calculated for all traits, based on both maternal and paternal full-sib families, as an estimate of its general combining ability (GCA).

Similarly, calculations were made of the SELF family means. For the field trial measurements, only OC families involving parents that produced a SELF family sufficiently large to be planted in the trial were included. For height and diameter growth after thinning in the field trial, calculations were made both for all replicates pooled and for each thinning treatment separately. For each parent the inbreeding depression was calculated within each replicate as the deviation between OC and SELF family means relative to the replicate OC family mean. It thus measures the relative reduction in performance of the SELF compared to the OC family, involving the same parents in all comparisons within each diallel. The total inbreeding depression of the trait was calculated as the mean value across all replicates. For height and diameter growth in the field trial, these calculations were made both based on all surviving trees and based only on trees that had a normal appearance.

All plot percentage values were transformed by the arc sine square root transformation and the statistical analyses were performed both on the percentages and on their transformed values. Only analyses based on percentages are presented as the conclusions were similar.

The main intention with this paper is to compare the performance of SELF with that of OC families from the same parents. Therefore a rather simplified statistical analysis will be presented here, and not a complete diallel analysis which will be presented elsewhere (SKRØPPA in prep.). The statistical model for trait Y measured on individual l in replicate k in the OC family between parents i and j (i#j) is assumed to be

$$Y_{ijkl} = \mu_o + G_{ij} + B_k + P_{ijk} + W_{ijkl}$$

where μ_o is the mean of the outbred population, G_{ij} is the combined genetic effect of the two parents, B_k and P_{ijk} are the block and plot effects, respectively, and W_{ijkl} is the residual error measuring both within-plot environmental and within-family genetic variability. All effects, except the mean, are assumed to be random with expectations zero. In the model for the SELF family of parent i, the index j should be substituted by i and the outbred population mean should be substituted by μ_s, which is the mean of the population of selfed individuals.

The difference between the OC and SELF family means from parent i in replicate k can be modelled by

$$D_{ik} = \mu_o - \mu_s + G_i + E_{ik}$$

where G_i is the genetic and E_{ik} is the residual component of the phenotypic difference, both effects being assumed random.

The inbreeding depression of SELF family i in replicate k is assumed to follow the model

$$ID_{ik} = \mu + F_i + H_{ik}$$

where μ is the mean inbreeding depression, F_i is a random genetic effect with mean zero and H_{ik} is a random error.

Both the hypothesis of no difference in mean between the outbred and selfed populations and the significance of the mean inbreeding depression were tested by t-tests in each diallel separately.

The significance of variation in performance among the SELF families in each diallel were tested by F-tests in two-way or three-way analyses of variance, based on percentage plot values for scoring traits or individual plant measurements for growth traits. The three-way analyses of the shoot elongation traits included family, year and replicate as the main effects and first and second order interactions. Synthetic F-tests were made to test the significance of the between-family variance component.

One-way (growth traits) and two-way (shoot elongation traits) analyses of variance were performed to test whether there is a significant variation in inbreeding depression among the SELF families.

Correlation coefficients were calculated between: (i) the means of SELF and OC families deriving from the same parent, and (ii) inbreeding depressions estimated at different ages for a same parent. Plots were made between the variables to verify the validity of these calculations.

Within-family variance components were calculated for SELF and OC families separately, based on mean variance components within each family plot. Variance differences between the family types were tested by Bartlett's test.

RESULTS

Population differences

The mean performance of the offspring from the three populations differed for several traits (Table 1). The trees of the Veldre diallel had on the average an earlier budset, a shorter growing season with a lower rate of growth and were consequently shorter than those from the two other diallels, which had similar mean values. This corresponds to provenance differences between altitudes 300 and 500 m. The parents in Diallels 2 and 3 were therefore pooled and will be presented as one group.

Performance and inbreeding depression of SELF families

The SELF families had a slower rate of germination and a lower final germination percentage than the OC families (Table 1). The mean inbreeding depressions for germination rate were of similar values and significantly different from zero ($p = 0.02$). For germination percentage, however, the inbreeding depression was rather low and significant only in Diallel 1. For seedling heights the first and second year, significant differences ($p < 0.001$) were present between the two family types, with the mean inbreeding depression varying between 0.23 and 0.31 ($p < 0.001$) (Table 1). The SELF families had a faster budset than the OC families.

The SELF families had a substantially higher mortality than the OC families after 5 years in the field test (total age 7 years) (Table 1). They also contained a higher proportion of trees with a non-normal development (dwarfs, bushy form, snake trees). The frequency of trees attacked by the gall aphids was considerably higher for the OC than for the SELF families. The SELF families had fewer branches in the whorl than the OC families. The differences between the OC and SELF families for these traits were significant at the 0.1% level. The assessments of lammas growth in the autumn of years 6 and 7 showed equal frequencies of trees with lammas shoots for SELF and OC families in Diallel 1, but a considerably higher frequency both years for the OC families in two other diallels combined, both with significant differences between the family types ($p < 0.001$).

The OC families were superior to the SELF families for all height, height increment and diameter measurements (Table 1). The differences between the two family types were significant at the 0.1% level for all measurements. The mean inbreeding depression estimates varied in the range from 0.23 to 0.41 and were all significantly different from zero. The inbreeding depression increased for heights from age 5 to 10. It was higher for the height increments of the years 6 and 7 than for those of the following three years, all replicates and thinning treatments pooled. These estimates were based on all surviving trees. Deleting those with a non-normal development lowered the inbreeding depression estimates with 0.02 to 0.04 units.

Table 1 Mean performance of selfed (SELF) and outcrossed (OC) families and mean inbreeding depression (ID) in Diallel 1 and Diallels 2 and 3 combined.

Trait	Unit	Diallel 1			Diallel 2 and 3		
		SELF	OC	ID	SELF	OC	ID
Germination rate	%	54.7	65.4	0.17	51.6	62.4	0.19
Germination percentage	%	85.4	92.2	0.07	90.0	94.2	0.05
Height year 1	mm	42	55	0.23	50	68	0.26
Height year 2	mm	143	197	0.27	175	251	0.31
Budset year 2	%	37.6	30.0	–	13.7	9.6	–
Mortality year 7	%	18.5	3.8	0.15	16.6	3.9	0.13
Non-normal tree form year 7	%	13.7	2.1	–	11.1	1.9	–
Gall aphid attack year 7	%	7.0	16.4	–	7.2	12.9	–
Lammas growth year 6	%	8.5	7.8	–	11.8	29.4	–
Lammas growth year 7	%	18.0	14.6	–	10.5	25.0	–
Number of branches year 6	#	3.9	4.5	–	4.0	4.8	–
Number of branches year 7	#	5.1	6.3	–	5.8	7.4	–
Height year 5	cm	30	41	0.28	38	50	0.23
Height year 7	cm	67	101	0.34	90	123	0.27
Height year 10	cm	141	211	0.34	180	265	0.32
Height increment year 6–7	cm	35	60	0.41	50	73	0.32
Height increment year 8–10	cm	74	108	0.31	97	138	0.30
Diameter year 10	mm	16	26	0.38	19	31	0.37
Wood density year 10	kg/m^3	405	383		406	384	
Growth initiation *)	day #	62.9	60.3	–0.06	61.5	59.6	–0.06
Growth cessation *)	day #	87.1	93.4	–	88.5	95.2	–
Duration of growth *)	days	24.2	33.1	–	27.0	35.6	–
Rate of shoot growth *)	mm/day	5.6	7.7	0.25 0.24	6.6	8.4	0.24 0.23

*) Mean of year 6 and 7

The inbreeding depression estimates of height, height increment and diameter after thinning differed only slightly between the thinning treatments.

The SELF families had significantly higher wood density than the OC families, resulting in negative inbreeding depression estimates. These differences are most likely effects of the slower growth of the SELF families and express correlated responses to the decrease in diameter growth.

The shoot elongation trait parameters presented (Table 1) are mean values of the 6th and 7th growth season. All differences between the two family types were significant at the 0.1% level. On average, the SELF families started their shoot elongation more than 2 days later than the OC families and terminated their growth 6–7 days earlier. Their shoot elongation period was therefore on average 8.5 days shorter than that of the OC families. The rate of shoot elongation was also lower for the SELF than for the OC families, with a difference of approximately 2 mm/day. The mean inbreeding estimates of the duration of the growth period and the rate of shoot growth were of the same magnitudes as for the height growth measurements. The reduced height growth of inbred Norway spruce trees with normal growth habit is therefore due to both a shorter growth period and a reduced growth rate.

Variation among SELF families

The variation among the SELF families within each group was highly significant ($p < 0.005$) for all height measurements. For mortality, tree form, gall aphid attack, lammas growth and number of branches in the whorl, significant variation ($p < 0.02$) was present among the SELF families in Diallels 2 and 3, but not in Diallel 1. The shoot elongation traits measured at ages 6 and 7 years showed no significant interactions between family and year. The variation among families was significant ($p < 0.01$) for the days of growth initiation and growth cessation ($p < 0.01$). The duration and rate of growth showed less variability among families ($0.002 < p < 0.15$).

The variation in inbreeding depression was highly significant for all height growth measurements and diameter in the combined Diallels 2 and 3 ($p < 0.01$). Less variability ($0.004 < p < 0.22$) was observed in Diallel 1. Fig. 1 presents the variation in inbreeding depression for three traits: total heights year 2 and year 10 and duration of the shoot growth period. The large variation in inbreeding depression in Diallel 2 is in particular due to parent 12 whose SELF progenies had an inbreeding depression lower than 0.10 for most traits.

Correlation patterns

The mean heights of SELF and OC families deriving from the same parents showed positive relationships in Diallel 1 (Table 2). In the other group of parents, however, there were no significant relationships between the heights of OC and SELF families (Table 2). Excluding parent 12, increased the correlations somewhat, and two of them became significant at the 5% level.

Table 2 Correlation coefficients between the mean heights of SELF and OC families at different ages for the two groups of parents. Significance at 5% and 1% level at values 0.67 and 0.80, and 0.60 and 0.74, respectively.

Age	Diallel 1	Diallel 2 and 3
1	0.83	0.51
2	0.93	0.30
5	0.78	0.52
7	0.63	0.09
10	0.73	0.21

Table 3 Correlation coefficients between inbreeding depressions of total height at different ages in the two groups of parents. Significance at 5% and 1% level at values 0.67 and 0.80, and 0.60 and 0.74, respectively.

Age	2	5	7	10
Diallel 1				
1	0.69	0.46	–0.06	0.25
2		0.78	0.10	0.15
5			0.61	0.58
7				0.73
Diallel 2 & 3				
1	0.97	0.89	0.81	0.68
2		0.94	0.88	0.79
5			0.92	0.85
7				0.92

Using the SELF families as a progeny test, all the three top ranked parents for final height would have been selected in Diallel 1, and only one of the three in Diallels 2 and 3.

In Diallel 1 there were no clear relationships between the relative self-fertility (SKRØPPA & THO 1990) and the inbreeding depression estimates of different traits. In the other group of parents, however, all correlation coefficients between the relative self-fertility and the inbreeding depression estimates of the growth traits were negative, varying from $r = -0.54$ ($p = 0.07$) to $r = -0.73$ ($p = 0.008$). When parent 12 was excluded,

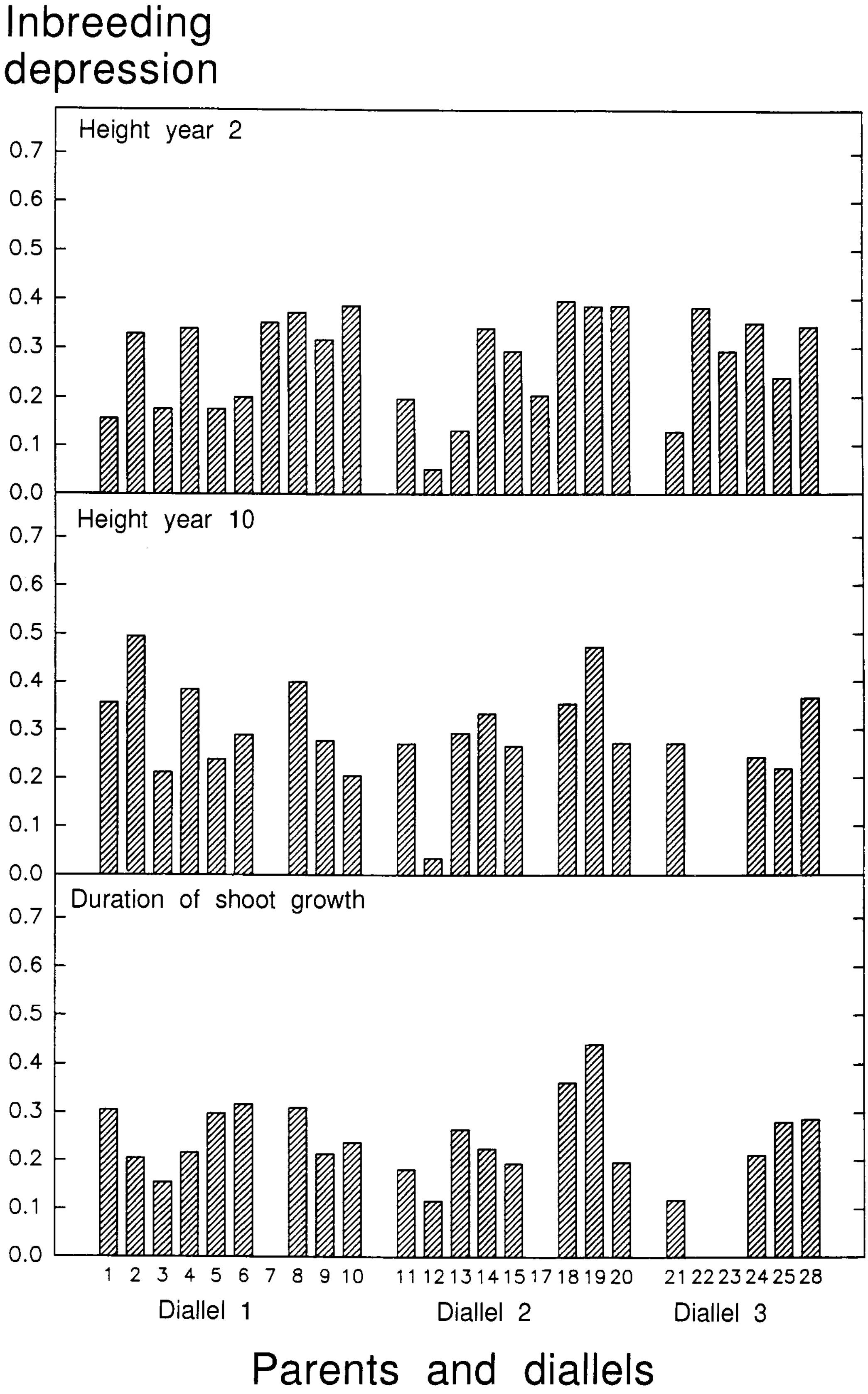

Figure 1 Variation in inbreeding depression for three traits: total height at ages 2 and 10 years and duration of the shoot growth period

the correlation coefficients were still nega-tive, but not significant, varying in the range –0.30 to –0.57.

The relationships among the inbreeding depression estimates of height at different ages were strong in Diallels 2 and 3 (Table 3). In Diallel 1, the correlations among the inbreeding depression estimates were weaker, and most were not significant.

These results suggest that different correlations patterns were present between the performance of the OC and SELF families and the inbreeding depressions in the two groups of parents. This was partly due to parent 12 which showed little inbreeding depression, but was still present even when this family was excluded from the calculations.

Variation within families

For seedling heights at the end of the first year there were only minor differences in within-plot variances between the OC and SELF families. For heights at year 2, however, the variances of the SELF families in the three diallels were 58%, 57% and 56% larger than those of the OC families. For height and diameter measurements in the field trial, the variances were in general larger for the SELF families, but of varying magnitude for the three diallels. In Diallel 1, the variances of the SELF families were on the average 8% larger than those of the OC families. In Diallels 2 and 3, the corresponding numbers were 42% and 30%, respectively. There was a slight tendency of a larger variability within the SELF families for the shoot elongation parameters as well, in particular for the duration of the growth period.

Even if there generally was a larger variance within the SELF than within the OC family plots, it was not possible for any of the traits to establish significant differences by Bartlett's test between the variance components of the two family types.

DISCUSSION

Methodology

The complete diallel crossing design is efficient for comparing SELF and OC families and estimating the level of inbreeding depression (LYNCH 1988). Except for a few traits in Diallel 2, the parental contribution was here balanced in all comparisons and a possible confounding of inbreeding depression and differential parental contribution (BURROWS & ASKEW 1982) was avoided. Balance in family sizes was also obtained initially in the short term field trial as only families with the sufficient number of seedlings were included. Some unbalance in family size could not be avoided in the later years, as the mortality was higher in the SELF than in the OC families. The level of selection was most likely different in the two family types when seedlings were chosen to be planted in the field trial. This was due to a lower number of surviving and vigorous seedlings in the SELF families in the nursery container plots. This may increase the difference between the two family types.

A possible pollen contamination may have large effect on the performance and within-family variability of SELF families. Such effects can never be completely excluded. In this experiment they are thought to be of minor importance. Isolation bags without pollinations were kept for control and yielded no filled seeds.

In the field trial, environmental variability was present both between replicates (blocks), between plots in the blocks and within each 4-tree plot. Attempts were made to minimize the influences of these factors as the statistical comparisons between SELF of OC families were made within the blocks and later averaged across all blocks. However, SELF family means estimates are less accurate than OC family means due to a lower number of SELF than OC plots. The experimental error in the calculation of the inbreeding depression is in particular due to the mean of the SELF family in each block which is based only on one plot of four trees, whilst the OC family mean is based on 6 (Diallel 3) to 16 (Diallel 1) times as many plots.

The parents in each diallel were selected as random trees at some distance apart in each stand subject to the condition that they should have both female and male flowers. They are therefore assumed to be a random sample from each population. However, only a small number of the parents in the stand is included and sampling effects may be substantial. Such effects must be taken into account when interpreting population differences.

Inbreeding depression in Norway spruce

The mean performance of SELF families was significantly different from the mean of OC families for almost all traits studied and in both parental groups. The SELF families germinated more slowly, had an earlier budset and a shorter and differently timed growth period with a lower rate of shoot elongation. As a consequence, the SELF families had a reduced growth performance for both height and diameter growths. In addition, they suffered a higher mortality and contained a higher frequency of trees with non-normal development. The mean inbreeding depression estimates varied for the different traits, but was non-significant only for germination percentage. The SELF families from the two altitudes 300 and 500 m showed similar levels of inbreeding depression.

These results correspond with the few inbreeding studies that have been performed in Norway spruce, which mostly have reported results for seed set, germination, height growth and volume production (ANDERSSON 1965; KOSKI 1971, 1973; ERIKSSON *et al.* 1973; ANDERSSON *et al.* 1974). They are also in good agreement with results from other conifer species (e. g. SORENSEN & MILES 1982; WILCOX 1983; FOWLER & PARK 1983; PARK & Fowler 1984, LUNDKVIST *et al.* 1987).

The mean inbreeding depression estimates of early height growth were highest in Diallels 2 and 3, while the opposite was the case for height growth in the field trial. For both groups the inbreeding depression of total height increased from age 5 to age 10. It was higher for height increments of the years 6 and 7 than for the next 3 years. These trends can first be related to increasing competition with age among trees at the narrow initial spacing, and secondly, to the effect of thinning. Inferior trees were then most likely removed at a higher frequency in the SELF than in the OC families. Similar increases in inbreeding depression by age, assumed to be caused by competition, have been shown in other studies (*e.g.* GEBUREK 1986; LUNDKVIST *et al.* 1987; SORENSEN & MILES 1982; see also UYENOYAMA *et al.* 1994). It has also been shown by DURELL *et al.* (1996) that inbreeding depression of annual height increments are more subject to fluctuations than total heights. The high inbreeding depression for diameter growth, which is assumed to be more effected by competition than height growth, is consistent with other studies (ERIKSSON *et al.* 1973; LUNDKVIST *et al.* 1987; DURELL *et al.* 1996).

Causes of inbreeding depression

There are two main hypotheses for the genetic basis of inbreeding depression in fitness traits (CROW & KIMURA 1970; LEDIG 1983; CHARLESWORTH & CHARLESWORTH 1987, FALCONER 1989). According to the overdominance hypothesis, the inbreeding depression is caused by some form of natural selection that favours the heterozygote at some loci. The dominance hypothesis, which is supported by most empirical data (LEDIG 1986; CHARLESWORTH & CHARLESWORTH 1987; STRAUSS & LIBBY 1987), explains the inbreeding depression by the effects of recessive or partial recessive deleterious genes whose effects are expressed in the homozyous recessive state. These genes are maintained in the population by mutation-selection balance (FALCONER 1989). Conifers, including Norway spruce, are known to carry unusual high levels of such mutants (KOSKI 1971, 1973; LEDIG 1986). In this material, the reduced fitness of the inbred Norway spruce families was in particular expressed by a reduction in filled seed percentages (SKRØPPA & THO 1990), an increased mortality in the field trial and a higher frequency of trees with a non-normal development compared to the OC families.

According to quantitative genetic theory, directional dominance is required for changing the population mean of a metric trait due to inbreeding (CROW & KIMURA 1970; FALCONER 1989). The change of the mean will be in the direction of the value of the more homozygous recessive individuals. In the presented results in Norway spruce a substantial and significant inbreeding depression was found for most traits. However, analyses of variance of the outcrossed families for the same traits revealed the presence of substantial amounts of additive genetic variances and very small non-additive genetic components (SKRØPPA, in prep.). These apparently conflicting results can be explained by the presence of recessive deleterious alleles occurring mainly in heterozygotes at low frequencies. After selfing, their effects will be expressed in homozygote recessive individuals and will influence the metric traits. Examples of this could be genes causing dwarfism or other deviating tree forms. This is a type of epistatic gene effects that are expressed only in homozygote recessive individuals, occurring most frequently in SELF families or after matings between close relatives. They will generally be expressed at very low frequencies in outcrossed families and can therefore not be detected as non-additive genetic variance in progenies after crossings among unrelated parents. The supposed correspondence between non-additive genetic variation and inbreeding depression can therefore not be taken for granted, and the existence of inbreeding depression for a trait cannot be used to predict non-additiv genetic variation. Similar results have been found for *Pinus radiata* by WILCOX (1983) and for *Eucalyptus grandis* by GRIFFIN & COTTERILL (1988), and are also well known in annual plants such as maize (HALLAUER & MIRANDA 1981).

A trait such as height growth may therefore be influenced by different types of gene actions when measured in SELF or OC individuals. It is therefore questionable whether such a character measured in the two types of families should be regarded as the same genetic trait. In any case, joint statistical genetic analyses for the estimation of genetic parameters combining data from both SELF and OC families should be avoided.

Variation in inbreeding depression

A large, and in the majority of cases, significant variation in inbreeding depression was found among the SELF families from each diallel. Similar variation has been found by GEBUREK (1986), LUNDKVIST *et al.*

(1987) and GRIFFIN & COTTERILL (1988). Such variability can be due to variation in the number of deleterious alleles carried by the different individuals. Causes can be random variation among individuals in the number of new mutations per generation or variation in the inbreeding history of the individual parents (SCHULTZ & WILLIS 1995). However, as shown by SCHULTZ & WILLIS (1995), alleles that act additively within loci may cause substantial variation in individual inbreeding depression estimates, even if the mean population inbreeding depression is zero. A variance in inbreeding depression for a quantitative trait may therefore not necessarily reflect any of the two causes stated above, but only be due to additive genetic variation. It is therefore not possible with the available data to identify the factors generating the variation in inbreeding depression of the quantitative traits in the three populations. It could be one or a combination of two or three of the factors: random variation in the number of deleterious alleles, individual differences in inbreeding history or merely reflect additive genetic variance.

Different correlation patterns among families were observed in the two groups. In Diallel 1, the mean heights at different ages of SELF and OC families were generally positively related, while the inbreeding depression estimates were not. In Diallels 2 and 3 combined, on the other hand, most individual inbreeding depression estimates, including the relative self-fertility, were strongly related. The variation in inbreeding depression was larger in this group. It is not possible to tell whether the observed differences between the two groups are due to sampling effects caused by the relatively low number of parents from each population or reveal population differences in the frequency of deleterious alleles and trait relationships.

Lethal and deleterious alleles may be identified by molecular genetic techniques when applied on individuals and their outcrossed and inbred offspring. Such studies will hopefully contribute to the understanding of individual and population differences in inbreeding depression.

Implications for breeding

A certain amount of selfing will always take place both in stands and in seed orchards of Norway spruce. For stands, it has been estimated that the proportion of self-fertilizations is around 10% in *Picea abies* (KOSKI 1973; MÜLLER 1977; XIE & KNOWLES 1994), but that only 1% of the filled seeds will originate from such matings (KOSKI 1973). In seed orchards, the selfing percentage may be higher and will depend on the design of the orchard. The presented results show that both survival, vitality and growth performance of selfed Norway spruce seedlings will be reduced in plantations. Efforts should therefore be made to reduce the selfing percentage in Norway spruce seed orchards both by choosing appropriate orchard designs and management regimes.

It has been proposed that SELF families after controlled self-fertilizations could be used in progeny tests to rank parents for general combining ability (WRIGHT 1980; HARDNER & POTTS 1995). A culling of inferior individuals within the selfed families prior to selection could be done to remove biases due to non-additive effects (BARKER & LIBBY 1974). The presented results show that this may not be a reliable method for progeny testing. Different types of genetic effects may influence the performance of individual SELF families, and they may not be ranked according to the additive genetic effects of the parents, even after a culling of the less vigorous individual (WILLIAMS & SAVOLAINEN 1996). A possibly larger within-family genetic variance of SELF than of OC families may not provide a higher genetic gain from within-family selection as the increased variance for a large part is due to non-additive genetic effects.

Selfing and milder forms of inbreeding have been suggested as tools in forest tree breeding (LINDGREN 1976). It has been proposed to make crosses among selected inbred lines for superior hybrid performance (DUREL & KRAMER 1995; WANG *et al.* 1996). Repeated selfing may be used to purge breeding populations for deleterious alleles, even if the efficacy of purging is uncertain (WILLIAMS & SAVOLAINEN 1996). When the inbreeding depression is as strong as for most traits in Norway spruce, one can assume that selfed offspring with lethal and highly deleterious alleles can be identified. Slightly deleterious alleles, however, may be more difficult to purge and may decrease fitness over generations (WILLIAMS & SAVOLAINEN 1996). Other critical factors in a breeding strategy based on selfing for Norway spruce are its long generation interval, the large population sizes needed, and, consequently, the high costs involved.

The purging of deleterious alleles may also be a selection for increased self-fertility (LINDGREN 1976), which may be unwanted both in seed orchards and in populations that may have to rely on natural regeneration in future generations. The inbreeding depression seems very likely to have played a part in the evolution of plant breeding systems (CHARLESWORTH & CHARLESWORTH 1987) and may be one of the factors explaining the maintenance of genic diversity within populations (LEDIG 1986). Intensive inbreeding and removement of the deleterious alleles from the reforestation populations may therefore have consequences for the future evolution of the species.

ACKNOWLEDGEMENTS

The diallel crosses were planned and performed by Jon Dietrichson. His large efforts to provide this unique genetic material are gratefully acknowledged. Thanks are also due to Thorleif Tho, who was in charge of the nursery part of the experiments, to all persons that have participated in planting and measuring the trials, to Øystein Johnsen for his comments to an earlier version of the paper, and to an anonymous referee for helpful comments. The diallel crosses were financed by the Agricultural Research Council of Norway.

REFERENCES

ANDERSSON, E. 1965: Cone and seed studies in Norway spruce (*Picea abies* (L.) Karst.). *Studia Forestalia Suecica* **23**:1–214.

ANDERSSON, E., JANSSON, R. & LINDGREN, D. 1974: Some results from second generations crossings involving inbreeding in Norway spruce (*Picea abies*). *Silvae Genetica* **23**:34–43.

BARKER, J. E. & LIBBY, W. J. 1974: The use of selfing in selection of forest trees. *Journal of Genetics* **61**: 152 –168.

BURROWS, P. M. & ASKEW, G. R. 1982: Experimental inbreeding depression. *Forest Sci.* **28**:148–156.

CHARLESWORTH, B. & CHARLESWORTH, B. 1987: Inbreeding depression and its evolutionary consequences. *Ann. Rev. Ecol. Syst.* **18**:237–268.

CROW, J. F. & KIMURA, M. 1970: An Introduction to Population Genetics Theory. Harper & Row, New York. 591 pp.

DUREL, C. E., BERTIN, P. & KREMER, A. 1996: Relationship between inbreeding depressionand inbreeding coefficient in maritime pine. *Theor. Appl. Genet.* **92**:347–356.

DUREL, C. E. & KREMER, A. 1995: Hybridization after self-fertilization:a novel perspective in the maritime pine breeding. *Forest Genetics* **2**:117–120.

ERIKSSON, G., SCHELANDER, B, & ÅKEBRAND V. 1973: Inbreeding depression in an old experimental plantation of *Picea abies*. *Heriditas* **73**:185–194.

FALCONER, D. S. 1989: Introduction to Quantitative Genetics. Third edition. Longman Scientific & Technical, England. 438 pp.

FOWLER, D. P. & PARK, Y. S. 1983: Population studies in white spruce I. Effects of self-pollination. *Can. J. For. Res.* **13**:1133–1138.

FRANKLIN, C. 1970: A survey of mutant forms and inbreeding depression in species of the family Pinaceae. *USDA Forest Serv. Res. Pap.* SE–61:1–21.

GEBUREK, T. 1986: Some results of inbreeding depression in Serbian spruce (*Picea omorika* (Panc.) Purk.). *Silvae Genetica* **35**:169–172.

GRIFFIN, A. R. & COTTERILL, P. P. 1988: Genetic variation in growth of outcrossed, selfed and open-pollinated progenies of *Eucaluptus regnas* and some implications for breeding strategy. *Silvae Genetica* **37**:124–131.

HALLAUER, A. R. & MIRANDA, J. B. 1988: Quantitative Genetics in Maize Breeding. Iowa State University Press, Ames.

HARDNER, C. M. & POTTS, B. M. 1995: Inbreeding depression and changes in variation after selfing in *Eucaluptus globulus* ssp. *globulus*. *Silvae Genetica* **44**:46–54.

KÄRKÄINEN, K. 1994: Deleterious mutations and sexual allocation in the evolution of reproduction of Scots pine. *Acta Universitas Ouluensis. A Scientiae Rerum Naturalium* **259**.

KÄRKÄINEN, K., KOSKI, V. & SAVOLAINEN, O. 1996: Geographic variation in the inbreeding depression of Scots pine. *Evolution* (in press).

KOSKI, V. 1971: Embryonic lethals of *Picea abies* and *Pinus sylvestris*. *Comm. Inst. For. Fenn.* **75**.3:1–30.

KOSKI, V. 1973: On self-pollination, genetic load and subsequent inbreeding in some conifers. *Comm. Inst. For. Fenn.* **78**.10:1–42.

LANGLET, O. 1940: Om utvecklingen av granar ur frö after självbefruktning och after fri vindpollinering. [Über die Entwicklung von teils nach künstlicher selbsbestäubung, teils nach freier Windbestäubung entstandenen Fichten]. *Medd. Statens Skogsförsanst.* **32**:1–22.

LEDIG, F. T. 1986: Heterozygosity, heterosis, and fitness in outbreeding plants. *In* : Conservation Biology. (ed. M. Soule) pp. 77–104. Sinauer Assoc. Inc., Sunderland, Massachusetts.

LINDGREN, D. 1976: Use of selfed materials in forest tree improvement. *Royal College of Forestry, Department of Forest Genetics, Research Notes* **15**:1–76.

LUNDKVIST, K., ERIKSSON, G., NORELL, L. & EKBERG, I. 1987: Inbreeding depression in two field trials of young *Pinus sylvestris* (L.). *Scand. J. For. Res.* **2**:281–290.

LYNCH, M. 1988: Design and analyses of experiments on random drift and inbreeding depression. *Genetics* **120**: 791–807.

MATHESON, A. C., WHITE, T. L. & POWEL, G. R. 1995: Effects of inbreeding on growth, stem form and rust resistance in *Pinus elliottii*. *Silvae Genetica* **44**:37–46.

MÜLLER, G. 1977: Untersuchungen über de natürliche Selbstbefrüchtung in Beständen der Fichte (*Picea abies* (L.) Karst.) und Kiefer (*Pinus sylvestris*). *Silvae Genetica* **26**:207–217.

OLESEN, P. 1971: The water deplacement method. *Forest Tree Improvement* (Arbor. Hørsholm) **3**:3–23.

PARK, Y. S., & FOWLER, D. P. 1984: Inbreeding in black spruce (*Picea mariana* (Mill.) B.S.P.: self-fertility, genetic load and performance. *Can. J. For. Res.* **14**: 17–21.

SCHULTZ, S. T. & WILLIS, J. H. 1995: Individual variation in inbreeding depression: The roles of inbreeding history and mutation. *Genetics* **141**:1209–1223.

SKRØPPA, T. & THO, T. 1990: Diallel crosses in *Picea abies*. I. Variation in seed yield and seed weight. *Scand. J. For. Res.* **5**:355–367.

SKRØPPA, T. & MAGNUSSEN, S. 1993: Provenance variation in shoot growth components of Norway spruce. *Silvae Genetica* **42**:111–120.

SORENSON, F. C. & MILES, R. S. 1982: Inbreeding depression in height, height growth, and survival of Douglas-fir, Poderosa pine and noble fir to 10 years of age. *For. Sci.* **28**:283–292.

STRAUSS, S. H. & LIBBY, W. J. 1987: Allozyme heterosis in radiata pine is poorly explained by overdominance. The *American Naturalist* **130**:879–890.

SYLVÉN, N. 1910: Om pollineringsförsök med tall och gran. [Über Selbstbestäubungsversuche mit Kiefer und Fichte]. *Medd. Statens Skogsförsöksanst.* **6**:219–228.

UYENOAMA, M. K., HOLSINGER, K. E. & WALLER, D. M. 1994: Ecological and genetic factors directing the evolution of self-fertilization. *Ox. Surv. Evol. Biol.* **9**:327–381.

WILCOX, M. D. 1983: Inbreeding depression and genetic variances estimated from self- and cross-pollinated families of *Pinus radiata*. *Silvae Genetica* **32**:89–96.

WANG, T. L., HAGKVIST, R. & TIGERSTEDT, P. M. A. 1996: Growth performance of hybrid families by crossing selfed lines of *Betula pendula* Roth. *Theor. Appl. Genet.* **92**:347–356.

WILLIAMS, C. & SAVOLAINEN, O. 1996: Inbreeding depression in conifers: Implications for breeding strategy. *Forest Science* **42**:102–117.

WRIGHT, A. J. 1980: The expected efficiencies of half-sib, testcross and S_1 progeny testing methods in single population improvement. *Heredity* **45**:361–376.

XIE, C. Y. & KNOWLES, P. 1994: Mating system and effective pollen immigration in a Norway spruce (*Picea abies (L.) Karst)* plantation. *Silvae Genetica* **43**:48–52.