Sitka Spruce Clonal Performance with Special Reference to Basic Density

23 Years' Results of a Clonal Trial

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Summary

The results from a 23 year-old Sitka spruce clonal trial are presented. Seven clones and a standard provenance were tested at one site. Significant differences between clones were found for the following characters at different ages from setting: height (age 3, 11, 23), diameter (age 11, 23), volume production (age 23), basic density (age 22), pilodyn (age 22), stem straightness (age 3, 23), form quotient (age 11) and volume level (age 23).

In order to compare the performance of the different clones in basic density, this trait was adjusted for differences in ring width using the latter as a covariate. It was shown that it is possible to select clones with both high basic density values and good growth.

The coefficient of clonal variation varied within a range from 5 % to 18 % for growth characters and 3 % to 14 % for quality traits at age 23 years.

Phenotypic correlations were calculated. At age 11, diameter seems to be a better indicator than height for volume production at later stages.

Key words: *Picea sitchensis*, clones, seedlings, basic density, growth characters, quality characters, clonal variation, phenotypic correlations.

FDC: 165.441; 232.11; 174.7 (*Picea sitchensis*).

1. Introduction

Sitka spruce (*Picea sitchensis* (Bong.) Carr.) is indigenous on the Pacific coast in North America, from Alaska down to northern California. It is an important exotic species in Ireland and in the United Kingdom. In France, interest in it has been increasing and in Denmark it is now one of the most-planted conifers.

In 1970, a breeding programme was started in Denmark (Brandt, 1970), which included a traditional seed orchard system with backward selection (i.e. selection among the clones according to their breeding value, based on progeny testing) and a system of selection and mass propagation by cuttings. The earliest Sitka spruce clonal trial in Denmark, and probably one of the earliest anywhere, was established in 1969 and 23 years' results are presented here.

Most expectations of gain from clonal forestry are based on results from young trials, where some clones may show high superiority, particularly in height growth. The latest results presented here are from an age close to half of the normal rotation age. Early results from this trial have been previously presented (Roulund, 1971, 1974, 1978; Roulund and Bergstedt, 1982).

2. Material and Methods

2.1. Plant material

The plant material consists of 7 clones and a population of seedlings.

The ortets were selected for height and stem form from an 8-year-old stand of Sitka spruce, in the "Rude Skov" forest, compt. 578 (Roulund, 1978). The provenance of this stand is "Wedellsborg F. 253", and the origin is presumably Washington.

The cuttings were set in 1969 at the Hørsholm Arboretum, according to the method described by Roulund (1971). The twigs (6 cm to 12 cm long), were taken all over the tree as shoots of second and third order and, before setting, they were mixed carefully. The intention was to simulate a practical situation and to see how much "variation associated with cloning", due to topophysis and size of the cuttings, could be expected in such a situation.

Unfortunately, no seedlings were available from the "Wedellsborg F. 253" provenance and, as a standard in this experiment, 2/0 seedlings from the selected stand "Rye Nørskov F. 229" (presumably also Washington origin) were used. This has been the standard in most Sitka spruce trials in Denmark, and so it can be used to link these clonal values to other genetic tests in the breeding programme of this species.

2.2. Experimental methods and designs

In the spring 1970, the rooted cuttings were transplanted to the nursery. The rooting percentage was 82.4 %. They were planted in a randomized block design, with 4 replications and 24 plants per plot. The spacing was 50 cm x 50 cm. In the autumn of that year, the same number of 2-year-old seedlings were transplanted to plots, which had been left empty within each replication.

In the spring 1973, the experiment was planted in the "Folehaven" forest in Hørsholm State Forest District. The area slopes moderately towards the northeast and there is a layer of humus (≈ 30 cm of thickness) over the moraine gravel. The experimental design was a randomized block design with 4 replications and 16 plants per plot, and the spacing was 2 m x 2 m.

A silvicultural thinning was made in the autumn of 1989, and 8 trees were left per plot.

2.3. The characters studied

The characters studied in this experiment are listed in *table 1*. They were measured on each standing tree at different ages from setting (i.e. from the time, in years, the cuttings were set and rooted).

Height was measured at the nursery stage at age 3 and in the field at ages 11 and 23. At the former stage, height was measured in units of 1 cm and it was defined as the length of the plant and not the vertical height of the apical bud (Roulund, 1978).

Diameter (1.3 m) measurements (over bark) were taken at ages 11 and 23.

Volume (total stem volume) was calculated for each tree at age 23, using the Danish standard volume functions for Sitka spruce (Madsen, 1987) and a Pascal programme developed by Madsen (1990). Two regression functions were

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Table 1. — Phenotypic mean values for several characters measured at different ages in seedlings and 7 clones of Sitka spruce. The basic density mean values were adjusted for ring width. The Adjustment was made separately for clones and seedlings, by using two different linear models.

Plant	Clone	Clone	Clone	Clone	Clone	Clone	Clone	Overall	Seedling
Character	V.3803	V.3805	V.3806	V.3807	V.3808	V.3809	V.3810	Clone Mean	Mean
Height (3 years) (dm)	6.97	5.69	6.26	5.50	5.64	3.99	6.91	5.85	3.85
Height (11 years) (dm)	54.48	52.68	54.06	53.78	46.35	47.43	59.94	52.67	42.13
Height (23 years) (dm)	182.76	190.77	181.79	191.83	177.07	165.34	190.30	182.84	161.93
Diameter (11 years) (mm)	64.72	50.34	54.05	66.51	66.65	53.52	69.57	60.77	44.91
Diameter (23 years) (mm)	204.27	194.35	180.68	224.85	201.61	182.26	210.39	199.77	163.53
Volume (23 years) (dm^3/tree)	302.33	286.16	242.43	397.09	301.63	229.33	347.31	300.90	179.38
Basic density (22 years) (kg/m^3)	326.4	350.5	335.5	289.1	317.3	311.1	349.7	325.7	350.0
Pilodyn (22 years) (mm)	22.37	23.62	20.69	27.94	25.25	25.06	19.00	23.42	20.66
Stem straightness (3 years)	1.96	2.50	4.26	2.89	2.60	3.60	3.30	3.02	3.02
Stem straightness (23 years)	5.78	6.09	5.44	5.65	4.00	4.19	5.62	5.25	5.72
Form quotient (11 years)	0.508	0.535	0.531	0.488	0.516	0.529	0.487	0.513	0.530
Volume level (23 years)	0.956	0.941	0.959	1.004	1.017	0.984	0.997	0.980	0.976

used: one general function based on total height, diameter 1.3 m and stand diameter (before thinning), and a similar function including also the diameter at 6 m above the ground.

Volume level is defined as ratio between the volume estimated from the function using the upper diameter (i.e. 6 m above the ground) and the volume calculated from the general standard function (MADSEN, 1990). Volume level was determined at age 23, and it gives a relative measure of the form factor or volume of the tree in question compared with a "standard" tree of the same height and diameter at breast-height.

The absolute *form quotient* is given by the ratio of the diameter half-way between 1.3 m and the top of the tree compared with the diameter at 1.3 m, both measured at age 11.

Basic density was assessed at age 22 years. Increment cores were taken through each tree at random, at an internodal position close to breast-height; 4 and 8 trees were randomly selected per plot for clones and seedlings, respectively.

The increment cores were then divided in segments, each containing 2 annual rings. Samples having knots, compression wood and other growth defects were rejected.

Due to the variation in time between trees in reaching breast-height, the years of formation of given rings also differ between trees. As reported by BRAZIER (1970), the substantial differences between average and maximum latewood densities in different years within both juvenile and mature wood of Sitka spruce, demonstrated the need for comparison of wood laid down in the same calendar year. Therefore, the samples were made up of the same ring numbers counted from the bark (i.e. they refer to comparable climatic periods). This selection procedure may introduce an error due to a comparison of annual rings of different age from the pith. However, this source of error is supposed to be small, as the differences in number of annual rings at breast-height were not pronounced within clones and seedlings (i.e. one annual ring in each of the cases). The calendar years of the ring segments are listed in *table 3*.

A variation in ring width may occur within each segment. This may result in an unequal contribution of each ring to the average basic density of the corresponding segment (OLESEN, 1976, 1982; HARVALD and OLESEN, 1987). Thus, a weighted average ring width of each segment was calculated, according to the width of each of the composing rings.

The volume of the green wood samples was determined using the water displacement method (OLESEN, 1971). The dry weights were measured after 24 hours of drying at 103 °C in an oven.

At age 22, the *pilodyn* wood tester (Proceq SA Zürich, Switzerland) was used to give also a measure of the wood density. In general, its mode of operation consists in injecting a springloaded steel pin into the wood, and the depth of penetration of the pin, which depends on the wood density, is given by a scale — with 0 mm and 40 mm as the lower and the upper limits, respectively (Proceq SA Zürich, 1985).

Following the same sampling procedure as for basic density, two pilodyn measurements were taken in opposite directions at breast-height of each tree.

At the end of the nursery period, *stem straightness* was scored visually according to a subjective scale made for description of plagiotropic growth (ROULUND, 1975). In this scale, 1 is straight and vertical (i.e. fully orthotropic growth) and 9 is bent and horizontal (i.e. fully plagiotropic growth). At age 23, another stem straightness scale was used. This scale has also values from 1 to 9, but here 9 is straight and 1 is very crooked. This has to be noted when both averages and correlations are considered in the comparison of characters.

2.4. Statistical methods

2.4.1. Basic density

In Sitka spruce, a negative phenotypic correlation has been found between wood density and ring width, where a high proportion of the variation in density can be accounted for by differences in ring width (BRAZIER, 1970; HARVALD and OLESEN, 1987; PETTY et al., 1990). The combined effect of a decrease in latewood percentage (i.e. an increase in earlywood width without a corresponding increase in latewood) and a reduction in the average density of the earlywood in the annual rings seems to be associated with the tendency referred above (BRAZIER, 1970).

Working with natural White spruce (*Picea glauca* (MOENCH) VOSS) populations, CORRIVEAU et al. (1987) showed a negative phenotypic correlation between wood density and the width of the annual rings.

In Norway spruce (*Picea abies* (L.) KARST.), a negative tendency in the interrelation between the 2 variables in question has also been reported (OLESEN, 1976, 1977; PETTY et al., 1990), and is mainly a result of the decrease in latewood percentage with increasing ring width (OLESEN, 1976, cit. BERNHART, 1964).

These studies suggest the necessity of recognizing the effects of ring width in order to compare different levels of a certain factor in basic density, assuming that the former variable reflects (at least partly) environmental conditions which affect the latter. Therefore, to remove the portion of the variation of basic density due to differences in ring width, the following analyses of covariance models with separate-slopes were applied:

2.4.1.1. Clones

$$Y_{ijk} = \mu + A_i + B_j + C_k + (AB)_{ij} + (AC)_{ik} + (BC)_{jk} + \beta_i RW'_{ijk} + e_{ijk}$$

where

Y_{ijk} = mean basic density of the kth genotype in the jth replication and ith two-year-period;

μ = constant;

A_i = *fixed* effect of the two-year-period i (i = 1 . . . 8) of ring formation;

B_j = *random* effect of the replication j (j = 1 . . . 4);

C_k = *random* effect of the genotype k (k = 1 . . . 7);

$(AB)_{ij}$ = *random* interactive effect of the period i and the replication j;

$(AC)_{ik}$ = *random* interactive effect of the period i and the genotype k;

$(BC)_{jk}$ = *random* interactive effect of the replication j and the genotype k;

RW'_{ijk} = mean ring width of the kth genotype in the jth replication and ith two-year-period (the mean of the transformation RW' = 1/(RW+2.0) was used — see below);

β_i = regression coefficient indicating the dependency of Y_{ijk} on RW'_{ijk} for the period i;

e_{ijk} = *random* error associated with the interactive effect of the period i, the replication j and the genotype k.

The adoption of this model was preceded by the following steps:

(i) Choice of the best covariate for controlling error variance

In Norway spruce, the interrelation between basic density and ring width ("Basic density level") was described mathematically by the hyperbolic function (OLESEN, 1976),

$$\text{Basic density} = a + \frac{b}{(RW + c)} = a + b\,(RW')$$

where a, b and c are constants, RW is the ring width and RW' is the transformed ring width (so that the theory of linear regression can be applied). In our case, RW is the weighted average ring width of each wood segment (i.e. each 2-year-period), as described in 2.3..

For the purpose in question, the transformed and untransformed ring width were compared by using an analysis of covariance model. Within the former, several values of the constant c (i.e. 0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0) were tried. The mean square of the error (MSE) after ad-

Table 2. — Basic density covariance analyses with separate-slopes for the 2-year-periods. The weighted average ring width of each 2-year-segment was transformed and then used as a covariate (see text). For effects indicated with an (a), the F-values and tests of significance are approximate and are based on the SATTERTHWAITE (1946) procedure.

Clones					Seedlings				
Sources of variation	Degrees of freedom	Mean squares	F_{value}	Probability of $F_{calc.} > F_{tab.}$	Sources of variation	Degrees of freedom	Mean squares	F_{value}	Probability of $F_{calc.} > F_{tab.}$
2-year-periods	7	410.79	4.98	< 0.001	2-year-periods	6	443.85	2.20	< 0.05
Replications (a)	3	990.11	1.39	> 0.1	Replications (a)	3	2912.56	0.92	> 0.1
Clones (a)	6	14016.97	14.23	< 0.001	Trees within Replications	27	2503.47		
2-year-periods * Replications	21	254.54	3.09	< 0.001	2-year-periods * Replications	18	897.76	4.44	< 0.001
2-year-periods * Clones	42	473.16	5.74	< 0.001	Ring width within 2-year-periods	7	7442.45	36.82	< 0.001
Replications * Clones	18	517.85	6.28	< 0.001	Error	147	202.10		
Ring width within 2-year-periods	8	1117.74	13.55	< 0.001					
Error	118	82.49							

justment for regression and the test of the null hypothesis about the partial regression coefficient, provided information on the value of the independent variable in controlling error and in increasing precision. The lowest MSE was obtained with the transformation RW' = 1/(RW+2).

(ii) Homogeneity of slopes

The test for homogeneity of regression coefficients was made separately for genotypes, replications and two-year periods of ring formation, by adding the interaction effects "genotype * RW' ", "replication * RW' " and "two-year-period * RW' " (respectively) to the model referred in step (i). The tests showed that the regression coefficients were approximately the same for all levels of the genotype and replication effects (Prob. > 0.05), but not for the two-year-periods (Prob. < 0.001).

In this experiment, the larger responses in basic density to changes in ring width were found for the second (1978 to 1979) and third (1980 to 1981) 2-year-periods, for both clones and seedlings. A larger basic density/ring width relationship was also shown by Harvald and Olesen (1987) in the inner rings of the juvenile wood of Sitka spruce. Therefore, it seems appropriate to use different regressions within each of the 2-year-periods (i.e. to use an analysis of covariance model with separate slopes for the 2-year-periods).

The F-values and tests of significance are represented in *table 2*. They were developed using the Type IV sums of squares from the SAS® PROC GLM. For the linear separate-slopes model applied here, the test of significance concerning the 2-year-periods effects has meaning given that ring width is held at its mean value. For replication and genotype effects, the F-values and tests of significance are approximate. Their calculations and the adjusted degrees of freedom are based on the Satterthwaite (1946) procedure.

The least squares or adjusted means were calculated for each level of both genotype and 2-year-period effects, and for the "genotype * 2-year-period" effects.

2.4.1.2 Seedlings

For seedlings, the following linear model was applied:

$$Y_{ijl} = \mu + A_i + B_j + T_{jl} + (AB)_{ij} + \beta_i RW'_{ijl} + e_{ijl}$$

where T_{jl} is the random effect of the tree l within the replication j and Y_{ijl} and RW'_{ijl} are the l^th^ tree observations on the 2-year-period i and replication j of the dependent variate and concomitant variable, respectively. The choice of this model was also preceded by the same steps as those for clones. The transformation RW' = 1/(RW+2) appeared also to be the best in controlling error variance. The tests of the homogeneity of slopes revealed significant differences (Prob. < 0.001) for "2-year-period * RW' ",but not for "replication * RW' " (Prob. > 0.05).

The F-value and test of significance for the replication effect are approximate and are based on the procedure referred above. The adjusted means were calculated for each level of both replication and 2-year-period effects.

2.4.2. Other characters

For each of the other traits, a 2-way analysis of variance based on plot means was performed, using the following linear random effects model:

$$Y_{jk} = \mu + B_j + C_k + e_{jk}$$

where Y_{jk} is the plot mean in replication j for genotype k and e_{jk} is the *random* error of the plot mean.

The coefficient of clonal variation was calculated for each trait and age from setting:

$$CV = \frac{\sigma_c}{\bar{x}} \cdot 100$$

where,

CV = coefficient of clonal variation;

σ_c = square root of the clonal component of variance;

$\bar{x}$ = overall clone mean.

3. Results and Discussion

3.1. Individual characters

3.1.1. Growth traits

On the average, clones perform better than the standard for growth characters measured at all ages (*Table 1*). For height and diameter, the relative mean difference between clones and seedlings is still substantial at age 23 (12.9% and 22.2 %, respectively), resulting in the large clonal superiority (67.7 %) for volume at this age and site. However, this difference between cuttings and seedlings may be misleading because provenance is not properly controlled in this experiment (the cuttings do not derive from the same population as the standard). Moreover, analysis of provenance experiments at age 27 years indicated that the seedlot of the seedling control "Rye Nørskov" performs better for height than "Wedellsborg F. 253", from where the clones were selected (Nielsen, 1993). The following reasons can be pointed out to explain the possible overestimation of the above mentioned superiority of the clones compared with the standard.

The mean height of the seedlings was considerably less than that of the clones at the time of planting in the field (Roulund, 1974, 1978). The clone V.3809 and the seedlings had similar rates of height development and approximately the same height at the time of planting (*Table 1*). This may be an indication that height at this stage of the experiment may be one of the reasons of the advantage of the cuttings compared with the seedlot. Besides, in the following years, frost damages (recorded as the frequency of broken leaders) had also a negative influence on height growth especially of the seedlings and the clone V.3809 (Roulund and Bergstedt, 1982).

Competition may have increased the differences in volume production in later years. A less diameter-dependent estimate of the volume differences can be made from a Danish yield table for Sitka spruce. Height differences of one meter correspond approximately to 45 m^3 (accumulated total volume production) per ha, assuming site class one and stand heights between 14 and 21 meters (Henriksen, 1958). For a fixed number of trees per ha, this leads to a superiority of approximately 57 % in volume production of the clones (18.3 and 16.2 meters being the overall clone and seedlot height means, respectively) compared with the seedlings at age 23 years. Therefore, competition seems not to be important.

On the other hand, the phenotypic selection intensities of the ortets for height (in the 8-years-old stand of Sitka spruce) varied from 2.74 to 4.34, corresponding to a selection differential of 57 % to 135 % for an overall stand height of 1.36 meters (Roulund, 1978). The correlations between these selection intensities and height and volume at age 23 years were respectively 0.79 and 0.73 (data not shown), which supports a successfull phenotypic selection. However, for a proper evaluation of the phenotypic selec-

Table 3. — Basic density, mean ring width and ring numbers from the pith for seedlings and clones of Sitka spruce. For each of the 2-year-periods of ring formation, the basic density mean values are adjusted for ring width. The ring numbers from the pith refer to the number of annual rings for the majority of the samples taken at breast-height.

Calendar years	Clones			Seedlings		
(years in ring segments)	Ring numbers from the pith	Basic density (kg/m^3)	Mean ring width (mm)	Ring numbers from the pith	Basic density (kg/m^3)	Mean ring width (mm)
1976 - 77	2-3	396.6	5.30	-	-	-
1978 - 79	4-5	371.2	7.72	3-4	430.9	6.22
1980 - 81	6-7	330.3	8.36	5-6	388.8	7.34
1982 - 83	8-9	311.5	6.35	7-8	351.5	5.25
1984 - 85	10-11	303.2	5.21	9-10	329.5	4.24
1986 -87	12-13	293.3	4.72	11-12	312.3	3.58
1988 -89	14-15	300.5	3.77	13-14	326.9	2.75
1990 - 91	16-17	298.6	4.46	15-16	309.8	2.94

tion efficiency, cuttings from a random sample of the base population could have been used instead of seedlings.

3.1.2. Quality traits

3.1.2.1. Stem straightness

No improvement in stem straightness is achieved by using this particular set of clones instead of seedlings (*Table 1*) at both nursery and field stages (at age 3 and 23, respectively).

Plagiotropic growth in the ramets is one of the consequences of the ortet maturation (Libby, 1974; Roulund, 1981). The plagiotropic growth observed at the nursery stage in three of the clones studied (Roulund, 1978), may have been the result of ageing in cuttings taken from the 8-year-old Sitka spruce ortets.

3.1.2.2. Basic density and pilodyn

The results presented in *table 2* show significant differences between clones and between the 2-year-periods of ring formation in basic density, after adjustment for ring width.

The age trend in mean basic density at breast-height (*Table 3*) shows a similar pattern of variation for both clones and seedlings. The basic density is very high in the first annual rings near the pith and then decreases until a minimum is reached at about ring number 12.

The interaction between clones and the 2-year-periods of ring formation is illustrated in *figure 1*. It shows also that, considering the behaviour of the individual clones, the variation of basic density with age follows the general pattern referred above.

The variation pattern shown is in agreement with that of other publications, concerning this character in Sitka (Harvald and Olesen, 1987) and in Norway spruce (Olesen, 1977), and it stresses the importance of ring number from the pith on density in juvenile wood. As reported by Olesen (1977), in the innermost rings, the number of tracheids per unit area decreases rapidly which will result in a strong decrease in basic density; however, with increasing ring number from the pith, this effect becomes

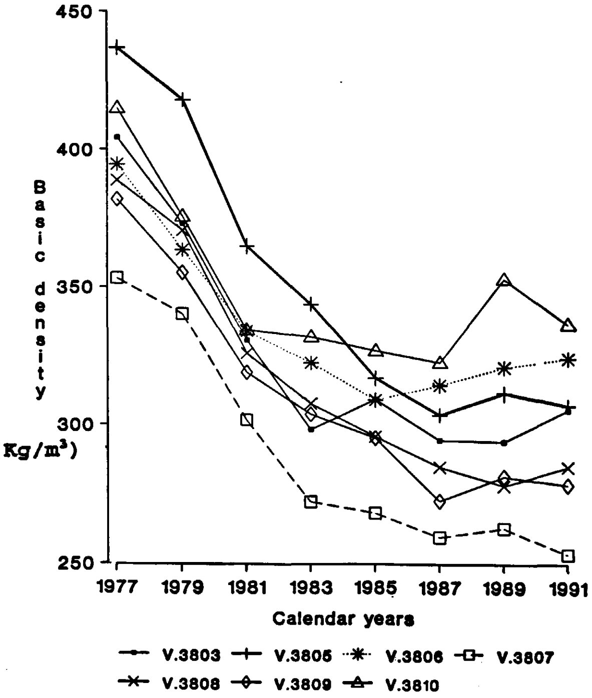

Figure 1. — Age trend in basic density in 7 clones of Sitka spruce. For each combination of clones and 2-year-periods of ring formation, the mean values of the basic density are adjusted for ring width. On the X axis, the second of 2 calendar years included within each period of ring formation is represented. The calendar years refer to the ring numbers from the pith indicated in *table 3*.

less pronounced and climate and latewood percentage become more important in changing the basic density level. In Sitka spruce, it seems that rings 8 to 15 constitute a transition zone in which both anatomical and climatic changes have a significant influence on the basic density level (Harvald and Olesen, 1987). This is in agreement with the results presented here.

The phenotypic correlation between basic density and pilodyn (data not shown) is high (i.e. —0.84). However, the general ranking between clones is slightly different for basic density and pilodyn (*Table 1*). This is particularly true for the case of the clone V.3805. The pilodyn measurements reflect the basic density of the outermost annual rings which, for the clone in question, is substantially reduced compared with its performance at earlier stages (*Figure 1*). This correlation between basic density and pilodyn, indicates a good opportunity for using a fast method for selecting between clones for the former character at 22 years. However, to judge the effectiveness of early selection using pilodyn, a correlation between the former and the whole basic density measured at later stages must be known.

3.1.2.3. Form quotient and volume level

The overall mean values for both form quotient and volume level (*Table 1*), suggest that the general behaviour of the clones does not differ from that of the seedlings but, for both factors, it seems that there are statistical significant differences between clones (*Table 4*).

3.2. Clonal variation

Differences between clone means are significant for the characters presented in *table 4*.

The high values of the coefficient of clonal variation for the characters measured at age 3 may reflect the existence of persistent non-genetic effects confounded with clonal genetic differences (i.e. "C" effects, following the terminology adopted by Libby and Jund, 1962). Inequalities between propagules — unequal size of the cuttings and different rhythms of plagiotropic to orthotropic

Table 4. — Analysis of variance and coefficient of clonal variation for 7 clones of Sitka spruce. The F-values and the significance of the difference between clone means are indicated for all traits (with the exception of basic density) at all ages from setting. Basic density was adjusted for ring width.

Character	F_{value}	Probability of $F_{calc.} > F_{tab.}$	Coefficient of clonal variation
Height (3 years) (dm)	15.09	0.0001	16.8
Height (11 years) (dm)	4.55	0.0056	7.7
Height (23 years) (dm)	7.15	0.0005	4.8
Diameter (11 years) (mm)	5.15	0.0031	11.6
Diameter (23 years) (mm)	5.70	0.0018	7.1
Volume (23 years) (dm^3/tree)	8.99	0.0001	18.2
Basic density (22 years) (kg/m^3)	-	-	6.6
Pilodyn (22 years) (mm)	34.37	0.0001	12.7
Stem straightness (3 years)	21.48	0.0001	24.8
Stem straightness (23 years)	6.39	0.0001	14.2
Form quotient (11 years)	3.58	0.0165	3.3
Volume level (23 years)	7.26	0.0005	2.7

Table 5. — Phenotypic correlations between several characters measured at different ages for 7 clones of Sitka spruce. The basic density mean values were adjusted for ring width. The significance levels of the correlations are indicated in parenthesis.

	Height (3 years) (dm)	Height (11 years) (dm)	Height (23 years) (dm)	Diameter (11 years) (mm)	Diameter (23 years) (mm)	Volume (23 years) (dm^3/tree)	Basic density (22 years) (kg/m^3)	Stem straightness (3 years)	Stem straightness (23 years)	Form quotient (11 years)
Height (3 years) (dm)										
Height (11 years) (dm)	0.75 (0.05)									
Height (23 years) (dm)	0.64 (0.12)	0.73 (0.06)								
Diameter (11 years) (mm)	0.48 (0.27)	0.32 (0.48)	0.30 (0.51)							
Diameter (23 years) (mm)	0.34 (0.45)	0.39 (0.39)	0.65 (0.11)	0.79 (0.03)						
Volume (23 years) (dm^3/tree)	0.36 (0.43)	0.46 (0.30)	0.73 (0.06)	0.75 (0.05)	0.99 (<0.001)					
Basic density (22 years) (kg/m^3)	0.50 (0.25)	0.46 (0.30)	0.27 (0.56)	-0.26 (0.58)	-0.36 (0.43)	-0.30 (0.51)				
Stem straightness (3 years)	-0.27 (0.56)	0.06 (0.90)	-0.28 (0.54)	-0.33 (0.46)	-0.51 (0.24)	-0.40 (0.37)	0.05 (0.91)			
Stem straightness (23 years)	0.61 (0.15)	0.79 (0.03)	0.83 (0.02)	-0.05 (0.91)	0.32 (0.48)	0.38 (0.40)	0.40 (0.37)	-0.21 (0.65)		
Form quotient (11 years)	-0.42 (0.35)	-0.55 (0.20)	-0.50 (0.25)	-0.90 (<0.01)	-0.89 (<0.01)	-0.89 (<0.01)	0.28 (0.54)	0.22 (0.63)	-0.22 (0.63)	
Volume level (23 years)	-0.20 (0.66)	-0.23 (0.62)	-0.14 (0.76)	0.70 (0.08)	0.48 (0.27)	0.47 (0.29)	-0.54 (0.21)	0.07 (0.89)	-0.61 (0.15)	-0.58 (0.17)

transitions — may have also increased both between- and within-clonal variance.

In this clonal trial, and in spite of the narrow range of clones included, there is an important clonal variation (with a range from 5 % to 18 %) for growth characters at age 23 years after setting. Useful variation between clones (with a range from 7 % to 14 %) is also found for basic density and stem straightness at age 22 and 23 years after setting, respectively. These features would result in gains from reselection on clone means, particularly for volume production and/or basic density at this stage of the experiment. However, the number of clones is not sufficient to provide reliable estimates of repeatabilities and gains from selecting between clones.

3.3. Phenotypic correlations

Early selection — which refers to indirect selection on a juvenile trait based on a correlated response with a mature trait (Nanson, 1969) — is a physiological requirement, when vegetative propagation is practised in a breeding programme. This is mainly due to the continued ortet and ramet maturation that occurs during the course of the clonal tests (Libby, 1974; Roulund, 1981; St. Clair et al., 1985). Besides the knowledge of time trends in clonal variances, repeatabilities and clonal juvenile-mature correlations must be determined to judge the effectiveness of early selection in terms of genetic gain per unit of time. Phenotypic clonal mean correlations are shown in *table 5* and they can be estimated only with large errors. Seven clones are an inadequate basis to calculate genotypic correlations and to use them to make inferences about the behaviour of a Sitka spruce population. Taking these aspects in account, the results presented in *table 5* can be discussed as follows:

3.3.1. Nursery period

For height, the early measurements at the end of the nursery period are well-correlated with this character at age 11 years. The correlation between height at the nursery stage and volume production at age 23 is not significant and the correlations between the former and height and diameter at later stages are declining with time.

The height growth of the clones V.3805 and V.3807 during the course of the experiment (*Table 1*), suggests that some clones overlooked at early ages may become outstanding at later stages. This can be an indication that early selection for height at the nursery stage may not be so effective at later stages and, particularly for volume production, this will probably result in an overestimation of the genetic gain.

The correlation coefficients between plagiotropism at age 3 and the other traits are not significant. In spite of the favourable relationship between stem straightness measured at age 3 and 23, it seems also that the former assessment is not so effective in predicting the performance for this character at later stages.

3.3.2. Field period

The correlation between heights at 11 and 23 years is slightly lower than that for diameters at these ages. Diameter at age 11 is reasonable, positively correlated with volume at age 23, and the correlation is much higher than the relationship between height and volume measured at these ages. Thus, clonal performance for diameter at 11 years after setting seems to be a good indication of performance in volume production at later stages.

Form quotient at age 11 years correlates negatively with volume level at age 23 years. This relationship is the opposite from what could be expected. High volume level indicates a high form factor, which should give also a higher diameter at half the distance between 1.3 m and total height. The main reason for the unexpected correlation is probably related to the dependence of the absolute form quotient on tree height (*Table 5*), whereas volume level seems to be more independent of tree size and, therefore, a more reliable measure of tree form.

The variation of basic density due to differences in ring width was taken into account by using the latter as a covariate. The negative relationship between basic density at age 22 years and diameter at 11 years is not significant and may be due to a random error effect. As useful clonal variation remains at age 22 years for the former trait, the results indicate that early selection for growth at age 11 and good performance in basic density at age 22 are not incompatible. The phenotypic correlation between basic density and volume was not significant. *Table 1* shows that clone V.3810 and, to a less extent, clone V.3803 can have simultaneously good growth and substantial basic density values.

4. Conclusions

The main strength of this experiment is its long period of testing, which is close to half the rotation age of Sitka spruce. However, its main limitation (due to the small number of clones tested) in providing reliable estimates of repeatabilities, clonal correlations and gains from reselection between clones restricts its application for inferences about the behaviour of a Sitka spruce population. Besides, this clonal test is established in only one location and, therefore, does not provide any information concerning the magnitude of possible genotype-environment interactions.

Taking in account the above aspects the 23 years' results of this Sitka spruce clonal trial show significant clonal differences, for both growth and quality characters measured at all ages. At age 3 years, the high values of the coefficient of clonal variation for height may reflect the existence of persistent non-genetic "C" effects. As mentioned by several authors (Libby and Jund, 1962; Libby, 1964; Burdon and Shelbourne, 1974), when vegetative propagation is practised some systematic non-genetic characteristics common to individuals of a given clone will probably appear and the total genotypic variance will be biased upwards. If these effects are important, the potentially great short-term gains made possible by large clonal variation in growth traits can be misunderstood (Shelbourne and Thulin, 1974). Nevertheless, in an experiment with 5-year-old Sitka spruce clones, Cannel et al. (1988) reported that the contribution of "C" effects (caused by the environment of the original ortets) to the total variation in height is small, compared with genetic effects.

The age trend in mean basic density at breast-height had a similar pattern of variation for both clones and seedlings from ring numbers 2-3 to 16-17 counted from the pith. The variation of basic density with age indicated that some changes in ranking can occur between clones.

The results show that it seems possible to choose clones with very favourable combinations of characters. The phenotypic correlation between basic density (after adjustment for differences in ring width) and volume was not significant, and it is possible to select clones for both high volume production and high basic density. Working with provenances of Sitka spruce, Murphy and Pfeifer (1990) concluded that large increases in vigour do not necessarily result in a decrease in density of the same magnitude, and considerable gains in growth rate can be achieved for a modest drop in wood density. Corriveau et al. (1987), also reported that in natural White spruce populations there were some trees and populations in which high wood density was associated with rapid growth.

Early selection seems to be possible for some of the traits, which is necessary for commercial cutting production before ageing of the ortets. Furthermore, it seems that there is a persistent growth superiority of the phenotypically selected clones compared with the seedling lot.

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Effect of Technique and Darkness on the Success of Meristem Micrografting of Picea abies

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Abstract

The possibility of micrografting *in vitro* shoot meristems of *Picea abies* were investigated on a 18 year-old Norway spruce clone. The rates of success were shown to be greatly influenced by the grafting technique used and by light, with a positive effect of a 2 to 3 week darkness period applied to the stocks just after they had been grafted. Average scores of more than 50% of success were obtained. However, substantial variability in terms of shoot expansion among the grafted plants existed *in vitro*, as well as after transfer to *ex-vitro* conditions.

Key words: micrografting, *Picea abies*, shoot meristem, technique, tissue culture, vegetative propagation.

FDC: 165.442; 174.7 Picea abies.

Introduction

There is much interest in favor of micrografting as broadly reviewed by Burger (1984) and Jonard (1986). With special regard to coniferous species, most of the work carried out in this field to date mentions the use of vegetative buds or shoot tips as scions (Misson and Giot-Wirgot, 1985; Tranvan and David, 1985; Ewald et al., 1991; Tranvan et al., 1991; Huang et al., 1992; Pullman and Timmis, 1992), but micrografting of shoot meristems has been restricted thus far to only a limited number of species (Monteuuis, 1986; Dumas et al., 1989; Goldfarb et al., 1993), despite the obvious benefits of miniaturizing the size of the grafted scion to the meristem. Meristem micrografting combines the advantages of grafting (Champagnat, 1980), with those of meristem culture, still problematic in practice for mature conifers (Pulmann and Timmis, 1992) to which it can constitute a helpful substitute. The possibility of introducing into tissue culture conditions contamination-free explants through grafted meristems derived from mature genotypes, while stimulating the potential for cloning of such introduced selected plant material at the same time (Franclet, 1983; Tranvan et al., 1991; Huang et al., 1992) must be considered a major argument for this technique. In addition, grafting meristematic tissues may help in reducing compatibility problems between the scion and the stock (Lachaud, 1975; Moore, 1984; Jonard, 1986).

The prospects of applying this attractive meristem micrografting technology to Norway spruce (*Picea abies* (L.) Karst.), a major forest species, were analysed and are reported in this paper.

Material and Method

Obtaining in vitro rootstocks

The *in vitro* seedlings used as rootstocks were obtained from *Picea abies* seeds that were surface-sterilized by immersion in 38 % hydrogen peroxide solution for 20 min, then rinsed 3 times in sterile distilled water before being individually inoculated into glass test tubes (25 mm x 200 mm) onto a 20 mm x 30 mm cellulosic "Sorbarod" plug (Baumgartner Papier SA, Lausanne. Switzerland). These Sorbarods had previously been saturated with 5 ml of liquid medium consisting of Margara (1977) macronutrients, Murashige and Skoog (1962) micronutrients diluted twice, 20 g/l sucrose and 10 g/l activated charcoal, before being autoclaved at 120 °C for 20 min.

The cultures were then maintained under a 16 h photoperiod with photon flux density for 110 μmol m^{-2} s^{-1} provided by "Sylvania Cool White" fluorescent lamps 36 W, and at 25/22 ± 2 °C, light/dark. Under these conditions, 50 % to 70 % of seeds that germinated developed within 2 to 3 months into young seedlings with fully expanded cotyledons and an elongating epicotyl that corresponded to the suitable stage to be grafted.

Scion origin

The apical meristems used as scions originated from vegetative buds produced by shoots of rooted cuttings of one AFOCEL superior clone of Norway spruce aged 18-years since seed germination. These 3-year to 4-year-old rooted cuttings were intensively maintained cultivated and hedged in large containers in a greenhouse with minimum temperature of 10 °C and permanent additional lighting provided by high-pressure sodium lamps.

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