

example 3. As the number of clones actually increases, the solution ought to be emotionally acceptable.

The term $\sum cp_i^2$ may be regarded as a "risk" factor or "disadvantage" factor. It is also closely related to the importance of genotype \times environment interaction. Preliminary calculations suggest that $\sum cp_i^2$ is not higher for the optimal unequal proportion solution compared to the equal proportion solution for the same c . Actually it may sometimes be a little lower. The algorithm suggest making greater use of the best clones, but also using more clones, than the conventional solution. The algorithm may be claimed to produce solutions with a higher net gain with no significant drop in safety, diversity or stability.

There are many factors not taken into account in this paper. We have assumed that the disadvantage of a clone due to its being more frequent is proportional to p_i with a constant proportionality factor c . But the relative disadvantage of clones may well vary. Data from *P. radiata* in Australia (MATHESON and GRIFFIN 1984 unpublished) suggests that inbreeding depression varies significantly between the same families as used in the example in this paper. This means that c is not a constant but a vector (c_i) instead. However the values of (c_i) are rarely known and it might be just as well to use some average figure. If the values are known with satisfactory accuracy, (c_i) may be used instead of c .

The contribution from different clones can be affected by phenological variation and could be modified by a factor to take this into account. The algorithm yields the optimum proportions of gametes of the clones and the actual clonal proportions should reflect variations in gamete production to achieve the desired proportions of gametes. In example 3 it is exemplified that some modification was done based on cone production.

The suggested algorithm depends on the proportion (p^*) being linearly dependent on the genetic values obtained experimentally. The estimates of genetic values are frequently not very reliable and this would certainly affect the values obtained for (p^*). However, given the data available, the formula proposed here probably still suggests the best proportions in the mix of clones in the seed orchard. If the information changes as time goes on, the orchard could be thinned towards a new "best" mix calculated from the new information. The algorithm suggested may be of great assistance when practising genetic thinning of existing seed orchards. The multiplier 0.04 is only an esti-

mate for a "typical" situation but if additional information is available another multiplier could be calculated, as shown in example 2.

In an advanced-generation breeding program there will be related candidates for seed orchards which would cause complications to our model. The question also arises for combined selection based on phenotype, progeny test plus information from other types of relatives. However, we would still recommend that the better clones be used in higher proportions even in this more complex situation. A particularly satisfactory feature of this algorithm is that it can be made to apply to multiple characters. Scores from any kind of selection index (expressed on a production scale) may be used instead of breeding values without loss of validity.

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Genotype-Environment Interaction and Stability in Ten-Year Height Growth of Norway Spruce Clones (*Picea abies* Karst.)

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Abstract

Norway spruce cuttings of 40 clones were tested on seven contrasting sites in northern Germany. Analysis of variance for ten-year height growth indicate a highly significant clone \times site interaction. This interaction may be reduced

by selection of stable clones. Several measures of stability were calculated and discussed. Characterization of sites by the method of genetic correlation indicate that most of the interaction is being generated between sites of high and low elevation. Stratification of the area into two planting

zones based on elevation would also reduce the interaction. Whatever method is used, the costs involved must be compared with the increase in genetic gain.

Key words: genotype \times site interaction, stability, Norway spruce clones, height growth.

Zusammenfassung

Stecklinge von 40 Fichtenklonen wurden auf 7 unterschiedlichen Standorten in Norddeutschland geprüft. Die Varianzanalyse für das Höhenwachstum im Alter 10 weist eine hochsignifikante Klon \times Anbaustandort Interaktion aus. Verschiedene Stabilitätsmaße wurden errechnet und verglichen. Beschreibt man die Standorte mit Hilfe der genetischen Korrelationen, so zeigt sich, daß die stärkste Interaktion zwischen Standorten unterschiedlicher Höhenlage auftritt. Eine Gliederung des Gebietes in zwei Anbauzonen aufgrund der Höhenlage, würde die Interaktion ganz wesentlich reduzieren. Ob man eine Gliederung des Anbaugebietes vornimmt oder Klone mit hoher Stabilität auswählt, muß aufgrund der Kosten im Vergleich zum Zuwachs im genetischen Gewinn entschieden werden.

Introduction

Genotype-environment interaction is the differential response of genotypes to changing environmental conditions. Such interactions complicate testing and selection in tree improvement programs, and result in reduced overall genetic gains. The literature on genotype-environment interactions is extensive. General reviews include COMSTOCK and MOLL (1963), ALLARD and BRADSHAW (1974), and FREEMAN (1973). Reviews within forest tree breeding include SQUILLACE (1970), SHELBORNE (1972), SHELBORNE and CAMPBELL (1976), MORGENSTERN (1982), and SKRØPPA (1984). MATHESON and RAYMOND (1984) evaluated the importance of genotype-environment interactions on *Pinus radiata* breeding programs in Australia using a criteria of the resulting loss of potential gain.

Genotype-environment interactions can be diminished in two ways: (1) by creating groups of essentially homogeneous environments and selecting cultivars suited to each environment, and (2) by developing stable cultivars which perform dependably over a range of environments. Stability may be achieved by population buffering and by individual buffering (ALLARD and BRADSHAW, 1964). Population buffering involves creating varieties composed of different genotypes adapted to a range of environments. It depends upon the elimination of less fit genotypes from the stand through intergenotypic competition. Individual buffering implies stable performance of individual genotypes and typically depends on heterozygosity, which implies a contribution of non-additive gene effects (ALLARD, 1961; ROWE and ANDREW, 1964; SCOTT, 1967).

Evidence from crop plants indicate that selection for stability may be effective (SCOTT, 1967). Non-additive gene effects, however, will be lost in a cycle of sexual reproduction, but may be captured clonal selection and propagation.

Clonal tree improvement programs may be designed to take full advantage of both individual and population buffering. Besides capturing both the additive and non-additive gene effects associated with stability, the composition and number of clones within clonal varieties can be chosen to provide maximum population buffering. In addition, clonal programs enable the development of varieties designed for smaller environmental units, something that is prohibitively expensive for seed-orchard tree improvement programs. Clones can also provide a more sensitive means

of detecting genotype-environment interactions and evaluating genotypic stability.

Norway spruce has proved to be a good species for large-scale clonal tree improvement programs. Cuttings from young trees root easily and show good growth and form (KLEINSCHMIT *et al.*, 1973; ROULUND, 1973, 1979). In addition, serial propagation appears to be effective in maintaining juvenility (St. CLAIR *et al.*, 1985). As a result several European countries have initiated operational clonal tree improvement programs using early selection and propagation of clones from provenance and progeny trials (KLEINSCHMIT and SCHMIDT, 1977; LEPISTÖ, 1977; ROULUND, 1977; WERNER, 1977; BENTZER, 1981; MONCHAUX, 1982).

As part of the clonal tree improvement program of the Lower Saxony Forest Research Institute, 40 clones were tested on seven contrasting sites in northern Federal Republic of Germany. This material provides an excellent opportunity to assess genotype-environment interaction and genotypic stability among clones used in an actual, ongoing tree improvement program. The objectives of this study are:

- (1) to examine the magnitude of genotype-environment interaction in ten-year height growth in Norway spruce clones;
- (2) to characterize these clones for stability;
- (3) to characterize sites in their contribution to the interaction;
- (4) to compare the merits of these sites as general testing environments;
- (5) to provide estimates of clonal repeatabilities and genetic gains for a Norway spruce tree improvement program.

Materials and Assessment

Propagation procedures and the breeding scheme of the Norway spruce program of the Lower Saxony Forest Research Institute have been described earlier in KLEINSCHMIT *et al.* (1973); KLEINSCHMIT (1974), and KLEINSCHMIT and SCHMIDT (1977). As part of this program, rooted cuttings are serially propagated on a three-year cycle. Selection based on nursery and field performance occurs at each repropagation. Cuttings of the 40 clones used in this study were tertiary cuttings (third cycle of vegetative propagation) rooted in spring 1974 and grown for three years in the nursery. The clones originated from different provenances of outstanding performance. The top provenance was Westerhof, therefore seedlings of a tested stand of this provenance serve as a base for comparison. The clones were selected at age 4 due to growth potential and entered clonal tests. According to the results of these clonal tests selection occurred between each repropagation. Therefore these 40 clones are the result of truncation selection. Clones are assumed not to differ in maturation. During spring 1977, cuttings were planted at seven contrasting sites in northern Germany (Figure 1). Heights to the 1983 whorl were measured with a height pole during summer 1984. Thus, height growth was after ten growing seasons, i.e. seven years after planting at the test site.

The seven sites were chosen to represent the range of sites in which Norway spruce clones from this program are expected to be planted. Three sites are located in the low coastal plains of northern Germany, while the other four sites are located in country of higher elevation further south (Table 1). The three low-elevation sites are warmer and drier than the higher-elevation sites. Medingen is the

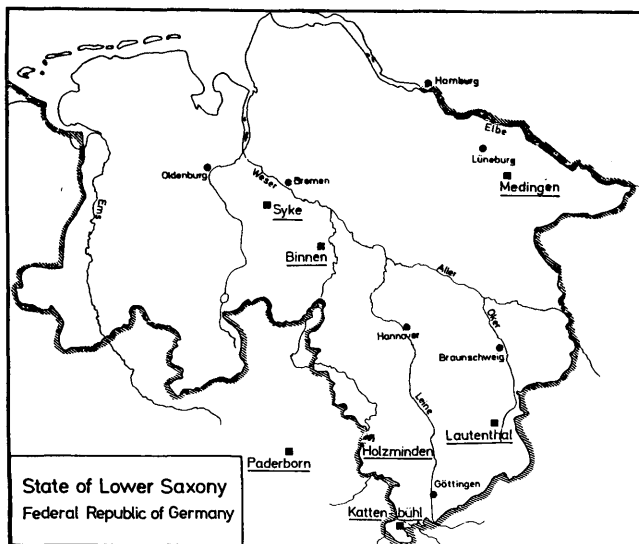


Fig. 1. — Map of northern Federal Republic of Germany showing location of tests sites as indicated by ■.

warmest and driest site, being further from the ocean than Syke and Binner. Lautenthal is located in the Harz mountains and is the coldest and wettest site. Soil and nutrient conditions were similar in the four higher-elevation sites, but vary from a nutrient-rich, loamy soil at Syke, to a less fertile site with a somewhat sandy soil at Medingen, to a nutrient-poor, sandy site at Binner.

The experimental design at each site was a randomized complete block design with 40 clones replicated in each of 20 blocks in single-tree plots. In addition to the 40 clones, each block contained nine 3-0 seedlings from the provenance Westerhof. Westerhof is considered an excellent provenance for planting in northern Germany (DIETRICHSON *et al.*, 1976), and may be used to evaluate gains achieved so far from clonal selection as compared to seedlings.

Statistical Analysis and Concepts

Analysis of variance was done for all sites combined using the statistical model

$$Y_{ijk} = \mu + C_i + S_j + CS_{ij} + \epsilon_{ijk} \quad (1)$$

where Y_{ijk} = ten-year height growth of the k^{th} ramet of the i^{th} clone at site j
 μ = overall mean
 C_i = effect of the i^{th} clone
 S_j = effect of the j^{th} site
 CS_{ij} = interaction between the i^{th} clone and the j^{th} site
 ϵ_{ijk} = error term.

Block effects within sites were not considered in this part of the study, owing to the unbalanced nature resulting from dead or missing trees. The form for the analysis of variance is given in Table 2. All effects were treated as random. Estimated variance components were used to calculate phenotypic variance, broad-sense heritability, repeatability of clonal means, and genetic gain. Formulas used include (see SHELBOURNE and THULIN, 1974):

Table 1. — Elevation, temperature and rainfall at seven test sites.

site	elevation (m)	mean temperature (°C)		mean rainfall (mm)		nutrient status	physical soil structure	comments
		year	growing season	year	growing season			
Syke	39	8.4	14.5	741	346	good	very good	Deep loam soil with good nutrient status
Medingen	50	8.5	15.6	606	296	mean	good	Sandy soil with some finer components
Binner	40	8.5	14.9	670	320	poor	poor	Sandy soil with poor nutrient status and poor drainage below 60 cm
Paderborn	340	7.8	13.8	1134	514	mean	very good	Loam soil with some sand components
Kattenbühl	350	7.5	13.3	800	380	mean	good	Sandy soil with loam upper horizon
Holzminster	445	7.5	13.4	900	420	mean	good	Sandy soil with some finer components
Lautenthal	575	5.9	12.0	1344	550	mean	mean	Loam soil with some sand components

Table 2. — Analysis of variance format for ten-year height of clones with sites combined (assuming fully random model).

source	df	expected mean squares
clones	c-1	$\sigma_e^2 + t\sigma_{cs}^2 + st\sigma_c^2$
sites	s-1	$\sigma_e^2 + t\sigma_{cs}^2 + ct\sigma_s^2$
clone x site	(c-1)(s-1)	$\sigma_e^2 + t\sigma_{cs}^2$
error	cs(t-1)	σ_e^2

Note: c = number of clones; s = number of blocks; t = effective number ramets per clone per site; σ_e^2 = variance due to differences among clones; σ_s^2 = variance due to differences among sites; σ_{cs}^2 = variance due to interaction of clones and sites; σ_e^2 = within-site variance.

$$\begin{aligned} \sigma_p^2 &= \sigma_c^2 + \sigma_{cs}^2 + \sigma_e^2 & (2) \\ \sigma_p^2 &= \sigma_c^2 + \frac{\sigma_{cs}^2}{s} + \frac{\sigma_e^2}{st} \\ h_B^2 &= \frac{\sigma_c^2}{\sigma_p^2} \\ R_c &= \frac{\sigma_c^2}{\sigma_p^2} \end{aligned}$$

where σ_c^2 , σ_{cs}^2 and σ_e^2 are estimated variance components (Table 2)

- σ_p^2 = phenotypic variance
- σ_{cs}^2 = phenotypic variance of clonal means
- h_B^2 = broad-sense heritability
- R_c = repeatability of clonal means
- s = number of sites
- t = mean number ramets per clone per site

Genetic gain was calculated in two ways (FALCONER, 1981). First, gain is calculated as $\Delta G = i\sigma_p^- R_c^-$ where i is the intensity of selection. In this study, it is assumed to equal 1.596 which corresponds to selection of five clones out of 40 (BECKER, 1984). Second, the selection differential D is determined from the difference of the mean of the top five clones and the overall mean. Genetic gain is then calculated as $\Delta G = D \cdot R_c^-$

As the clone \times site variance component increases relative to clonal variance, the repeatability of clonal means decreases, and thus, genetic gains are reduced. One solution is to select clones with high stability. Several statistical techniques have been proposed to characterize stability.

The most common approach, known as joint regression analysis, regresses the yield of each genotype upon some environmental index (FINLEY and WILKINSON, 1963; EBERHART and RUSSELL, 1966; PERKINS and JINKS, 1968; SKRÖPPA, 1984). In terms of the model presented above:

$$CS_{ij} = \beta_i I_j + \delta_{ij} \quad (3)$$

where β_i = departure of the linear regression coefficient of the i^{th} clone from the overall linear regression coefficient
 I_j = environmental index of the j^{th} site
 δ_{ij} = deviations from the regression line of the i^{th} clone at the j^{th} site.

If the environmental index is taken to be the mean yield of all genotypes in that environment, then I_j becomes the site effect S_j and the model can then be rewritten as:

$$Y_{ijk} = \mu + C_i + (1 + \beta_i) S_j + \delta_{ij} + \epsilon_{ijk}$$

The interaction sums of squares can then be partitioned into two components, the sum of squares due to differences in individual regression lines with (c - 1) degrees of free-

dom, and the sum of squares due to deviations from the regressions with (c - 1) (t - 2) degrees of freedom (FREEMAN and PERKINS, 1971).

FINLEY and WILKINSON used the estimated regression coefficient b_i (where b_i estimates $1 + \beta_i$) to measure stability and relative adaptability. A variety with a value near one was considered to be of average stability and equally adapted to good and poor sites. A variety with a value greater than unity was of low stability and better adapted to good sites. A variety with a value less than unity was of high stability and better adapted to poor sites. EBERHART and RUSSELL (1966) proposed the use of an additional stability parameter, the mean squares deviations from the regression for each genotype, as measured by S_{di}^2 ; a stable variety was defined as one with $b_i = 1.0$ and $S_{di}^2 = 0$.

The use of the mean of all genotypes as the environmental index has been criticized on statistical grounds (FREEMAN and PERKINS, 1971; HARDWICK and WOOD, 1972). However, provided that the numbers of genotypes and environments are reasonably large and the environmental range is sufficient, linear regression using the mean of all genotypes should be biologically valid (FRIPP and CATEN, 1971; FRIPP, 1972; HÜHN, 1980; SKRÖPPA, 1983).

The technique of FINLEY and WILKINSON (1963) and EBERHART and RUSSELL (1966) is used in this study to characterize genotypic stability. In addition, WRICKE'S (1962) ecovalence and S_{ij} from HÜHN (1979) are calculated. Ecovalence is the contribution of each genotype to the interaction sums of squares, and is given by $\sum_j (Y_{ij} - \bar{Y}_i - \bar{Y}_j + \bar{Y} \dots)^2$, a low value indicating greater stability. S_{ij} is the average change in rank between all pairs of environments for genotype i, that is $S_{ij} = \frac{2}{n(n-1)} \sum_j |r_{ij} - r_{ji}|$. It is one of six

stability parameters based on rank changes as developed by HÜHN. A genotype which ranks similarly in different environments is considered stable and has a low value for S_{ij} .

The contribution of environments in generating interactions was studied using a method developed by BURDON (1977). He considers heights in different environments as separate traits and calculates the genetic correlations between heights at pairs of environments. Gain for selection at one site with planting at another is determined from the formula for correlated response to selection (FALCONER, 1981). Estimated correlations can then be used to indicate which sites are most similar to one another (in terms of showing least interaction), and a matrix of expected gains can indicate which sites are best for testing.

The formula for expected gain at site y based on clonal selection at site x can be written as:

$$\Delta G_{y,x} = i \sqrt{R_{cx}^-} \sigma_{cy} r_{xy} \quad (4)$$

where i = intensity of selection

R_{cx}^- = repeatability of clonal means at site x

σ_{cy}^2 = clonal component of variance at site y

r_{xy} = correlation between clonal means at sites x and y.

Variance components and repeatability of clonal means were estimated from analyses of variance done separately for each site. The statistical model considered effects due to clone, block and error. Clone \times block interaction was confounded with the error term since single-tree subclasses were used. All twenty blocks were included.

Calculations were carried out in Göttingen on the UNIVAC-computer 1110 of the "Gesellschaft für wissenschaftliche Datenverarbeitung" and on the TA 1600/30 of the Lower Saxony Forest Research Institute, using SPSS, Harvey and own programs.

Result and Discussion

Overall means and analysis of variance

All effects, including clone × site interaction, where highly statistically significant ($p < 0.001$) in the overall analysis of variance (Table 3). Site means decreased with increasing elevation and decreasing temperatures (Table 4). Syke was the top-ranked site with a mean ten-year height of nearly three meters, 2.3 times better than the bottom-ranked site, Lautenthal. Differences between the three low-elevation sites may be related to soil structure and nutrient availability.

Table 3. — Analysis of variance for ten-year height (meters) of 40 clones at seven sites.

source	df	MS	F	variance components	
				magnitude	%
clones	39	6.52	9.08***	0.048	13.0
sites	6	209.91	292.29***	0.305	82.0
clone × site	234	0.72	1.80***	0.019	5.0
error	4524	0.40		0.399	

Note: *** indicates highly significant, $p < .001$; variance component percentage is from total excluding error variance.

Overall clonal means ranged from 1.66 meters for Clone 189 to 2.69 meters for Clone 37. Clone 37 was particularly outstanding, ranking first at five out of seven sites.

The significant clone × site interaction indicates that clones perform differently between sites. Of greater in-

Table 4. — Mean 10-year heights for 40 clones and seedlings of provenance Westerhof at seven sites (in meters).

	Syke		Medingen		Binnen		Paderborn		Kattenbühl		Holzminden		Lautenthal		overall	
clone	mean	rank	mean	rank	mean	rank	mean	rank	mean	rank	mean	rank	mean	rank	mean	rank
37	3.71	1	3.29	2	2.77	6	2.56	1	2.50	1	2.16	1	1.81	1	2.69	1
123	3.52	6	3.36	1	3.04	2	2.37	3	2.46	2	2.12	2	1.55	2	2.63	2
95	3.51	7	2.91	6	3.01	3	2.22	8	2.29	5	1.66	15	1.44	7	2.43	3
41	3.52	5	3.02	4	2.87	4	2.11	15	1.89	23	1.97	3	1.44	8	2.40	4
107	3.59	2	2.82	9	3.05	1	2.20	9	1.98	16	1.71	10	1.09	33	2.35	5
143	3.02	20	2.51	20	2.83	5	2.26	7	2.44	3	1.86	5	1.35	13	2.32	6
103	3.49	8	2.62	14	2.59	8	2.01	23	2.36	4	1.75	9	1.33	16	2.30	7
152	3.55	3	2.86	8	2.15	19	2.41	2	2.12	7	1.65	17	1.23	25	2.28	8
188	3.20	10	3.02	3	2.22	13	2.28	5	2.10	8	1.62	23	1.47	4	2.27	9
197	3.53	4	2.76	10	2.65	7	2.26	6	1.82	28	1.47	30	1.31	17	2.26	10
125	2.78	27	2.67	13	2.38	10	2.08	16	2.06	10	1.87	4	1.47	6	2.19	11
45	3.20	11	2.46	24	2.44	9	1.92	27	1.96	18	1.81	7	1.50	3	2.18	12
26	3.19	12	2.86	7	2.15	18	2.08	17	2.04	11	1.66	16	1.31	18	2.18	13
101	3.10	14	2.92	5	2.40	10	1.96	25	1.89	24	1.46	32	1.25	22	2.14	14
46	3.37	9	2.61	15	2.18	16	1.79	34	2.26	6	1.43	34	1.15	31	2.11	15
90	2.91	21	2.50	22	2.00	27	1.98	24	1.98	15	1.84	6	1.38	12	2.08	16
87	3.09	16	2.68	12	2.06	23	1.89	28	1.98	14	1.69	12	1.20	28	2.08	17
50	3.15	13	2.57	18	2.21	14	2.04	20	1.72	34	1.69	13	1.08	35	2.06	18
18	2.90	22	2.71	11	2.09	20	2.02	21	1.74	33	1.62	20	1.35	14	2.06	19
42	3.09	17	2.58	16	1.78	33	2.02	22	1.89	22	1.81	8	1.19	29	2.05	20
142	2.83	24	2.38	26	2.36	12	2.07	18	1.75	32	1.63	19	1.23	26	2.04	21
9	2.79	26	2.31	31	2.04	24	2.35	4	1.85	27	1.59	25	1.29	19	2.03	22
4	3.09	15	2.21	34	2.08	21	1.82	32	2.06	9	1.62	20	1.26	20	2.02	23
145	3.04	18	2.27	32	1.68	37	2.11	14	1.94	19	1.70	11	1.38	11	2.02	24
15	2.69	31	2.58	16	1.91	30	2.04	19	2.01	13	1.47	31	1.34	15	2.01	25
88	2.54	36	2.34	28	2.15	17	2.13	13	1.93	20	1.61	24	1.25	23	1.99	26
118	2.75	28	2.47	23	1.86	32	1.80	33	1.86	26	1.64	18	1.47	5	1.98	27
115	2.55	35	2.33	30	1.91	28	2.17	10	2.03	12	1.51	27	1.24	24	1.96	28
113	2.80	25	2.51	21	1.47	40	2.16	11	1.97	17	1.37	36	1.43	10	1.96	29
94	2.73	30	2.54	19	1.68	38	2.14	12	1.72	35	1.68	14	1.09	34	1.94	30
98	2.75	28	2.12	37	2.06	22	1.96	26	1.88	25	1.56	26	1.03	37	1.91	31
116	3.03	19	2.45	25	2.02	26	1.71	37	1.77	31	1.33	38	1.02	38	1.90	32
104	2.48	37	2.13	36	1.91	29	1.84	30	1.92	21	1.62	22	1.17	30	1.87	33
196	2.48	38	2.05	38	2.19	15	1.88	29	1.78	29	1.45	33	1.21	27	1.86	34
181	2.69	32	2.34	29	1.76	35	1.83	31	1.69	37	1.47	29	1.05	36	1.83	35
173	2.88	23	2.35	27	1.87	31	1.79	35	1.64	38	1.43	34	0.86	40	1.83	36
66	2.60	33	2.22	33	1.68	36	1.58	38	1.69	36	1.32	39	1.43	9	1.79	37
112	2.57	34	2.01	39	1.64	39	1.56	39	1.78	30	1.35	37	1.15	32	1.72	38
11	2.19	40	2.16	35	2.03	25	1.73	36	1.55	39	1.24	40	0.92	39	1.69	39
189	2.42	39	1.86	40	1.77	34	1.53	40	1.30	40	1.51	28	1.26	21	1.66	40
overall clonal means	2.97		2.54		2.18		2.02		1.95		1.63		1.28		2.09	
seedling means	2.24		2.09		1.84		1.74		2.22		1.41		1.08		1.81	

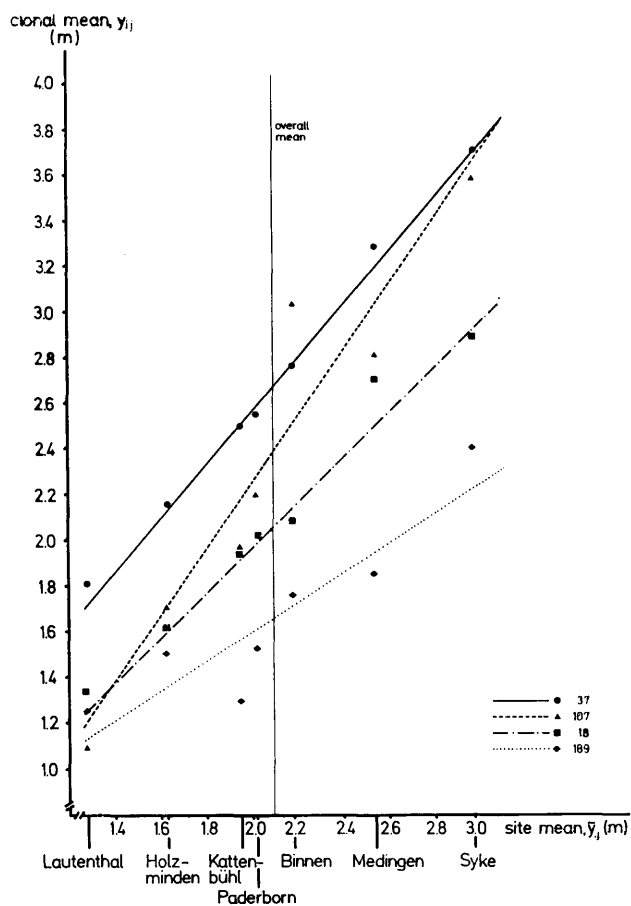


Fig. 2. — Plot of clonal means for four clones against site means with corresponding regression lines.

terest than statistical significance is the importance of the interaction in reducing gains. The magnitude of the interaction component was less than half that of the clonal variance component (Table 3).

Broad sense heritability (h^2_B) overall for 10-year height growth was 0.10, which is relatively low. However, repeatability of clonal means (R_c) overall was 0.89. The genetic gain based on a selection intensity of $i = 1.596$ is 0.33 m, a gain of 16 percent above the overall mean. If the top five clones are selected and genetic gain calculated based on the observed selection differential, the genetic gain is 0.42 m, 20% above the overall mean. The difference between the two gain estimates results from the outstanding height growth of clones 37 and 123. These gain estimates represent gains to be achieved from further clonal selection, and do not include gains already achieved from past selection.

Stability

The F-value for heterogeneity of regressions from the breakdown of the interaction sums of squares confirms the statistical significance of the clone \times site interaction (Table 5). Values for the regression coefficient b_i range from

Table 5. — Breakdown of interaction sums of squares.

Source	df	MS	F
heterogeneity of regressions	39	1.42	3.56***
deviations from regressions	195	0.58	1.44**

Note: *** $p < .001$; ** $< .01$.

0.65 to 1.46 (Table 6). The plot of clonal means against site means with the corresponding regression lines for four clones illustrates the differential reactions of clones to changing environments (Figure 2). Clone 18 represents a clone of average stability ($b_i = 0.99$) as defined by FINLAY and WILKINSON (1963). Its performance is relatively equal on poor and good sites. Clone 189 represents a clone of high stability ($b_i = 0.65$). It performs relatively better on poor sites (although its overall performance is poor). Clone 107 represents a clone of low stability ($b_i = 1.46$). It performs relatively better on good sites.

The point is, the regression coefficient measures *relative* performance. In forest tree breeding this information is useful to distinguish genotypes for specific environments, but if all environments tested are in one planting zone, and each represents the same proportion of area to be planted, then this information is irrelevant. Selection on the overall mean is all that is necessary to assure the largest overall gains.

More important to forest tree breeding is the predictability of yield of a genotype in various environments. This concept of stability may be measured by the mean deviations from the regression line, S^2_{di} (BECKER, 1981). Clone 107 performs well overall, but is very unstable as measured by S^2_{di} (Table 6). Clone 37 also performs well, but is very stable as measured by S^2_{di} . This is apparent from the deviations of the individual points from the regression line in Figure 2.

The conclusions are the same when the other stability parameters, ecovalence and S_{ij} , are considered (Table 6). Clone 37 rates as very stable, and Clone 107 as unstable. Rank correlation between ecovalence and S^2_i is high, and is still good between S_{ij} and S^2_{di} , and S_{ij} and ecovalence (Table 7).

A plot of the stability parameter against the clonal means is useful as an aid to selection (Figure 3). In each figure, clones falling in the lower, right-hand side are preferred. Clone 37 is the best clone no matter which stability parameter is used. Selection of clones based on height and stability can then proceed using a selection index for multiple-trait selection (SONECYPHER and ARBEZ, 1976), but assigning weights based on relative economic value may prove difficult. This would require knowledge of the reduction in gain associated with using unstable versus stable genotypes. The use of independent culling levels, that is, setting an acceptable value for a stability parameter followed by selection based on height, may prove more practical.

Correlated gains among sites

The expected gains from various combinations of testing and planting sites, along with estimates of clonal variance and repeatability of clonal means are presented in Table 8. Expected gains at planting sites are generally greatest or close to the greatest, when selection is done at the same site. Where this is not true (Paderborn, Kattenbühl, and Holzminden), the clonal variance is small relative to that at Syke, Medingen, and Binnen. Selection based on overall means results in gains second only to those when selection is done at the same site as planting. When selection is done at a single site for planting at all sites, the best sites for testing, as indicated by the largest gains, were Medingen, Syke and Binnen. These three sites were of higher site index, and had the largest value for repeatability of clonal means and the greatest clonal variances.

Medingen and Syke were the best single sites for testing, largely because of the high correlations with most of the

Table 6. — Stability statistics for 10-year height growth.

clone	regression coefficient b_i	rank	mean square dev. S_{di}^2	rank	ecovalence	rank	S_{1j}	rank
37	1.16	12	0.01	1	0.06	2	1.6	1
123	1.22	8	0.20	31	0.30	30	1.6	1
95	1.28	5	0.28	35	0.43	36	4.1	4
41	1.26	6	0.31	37	0.44	37	8.2	15
107	1.46	1	0.40	39	0.80	40	11.9	35
143	0.92	24	0.36	38	0.37	34	8.5	17
103	1.21	9	0.20	30	0.28	29	7.5	11
152	1.34	3	0.12	21	0.34	32	10.6	29
188	1.14	15	0.12	20	0.15	15	7.9	13
197	1.38	2	0.21	33	0.48	38	12.6	37
125	0.80	33	0.05	7	0.13	11	8.8	19
45	0.97	20	0.14	23	0.15	14	11.0	31
26	1.16	13	0.05	5	0.09	7	5.0	6
101	1.23	7	0.12	19	0.22	23	11.4	34
46	1.30	4	0.19	29	0.36	33	14.4	40
90	0.86	29	0.06	9	0.09	6	9.0	20
87	1.11	16	0.06	8	0.08	4	8.5	17
50	1.19	11	0.07	10	0.13	12	10.9	30
18	0.99	18	0.90	12	0.09	5	7.9	13
42	1.04	17	0.21	32	0.21	22	10.0	27
142	0.94	23	0.10	16	0.11	10	7.9	13
9	0.86	30	0.17	27	0.20	21	9.4	24
4	0.98	19	0.17	28	0.17	16	10.5	28
145	0.88	26	0.30	36	0.32	31	11.9	35
15	0.88	27	0.12	18	0.14	13	9.5	25
88	0.77	36	0.08	11	0.18	19	9.1	22
118	0.79	34	0.09	14	0.17	18	11.2	33
115	0.78	35	0.14	22	0.24	26	11.0	31
113	0.87	28	0.50	40	0.53	39	14.1	39
94	0.94	22	0.27	34	0.28	28	12.9	38
98	0.92	25	0.09	13	0.10	8	6.8	8
116	1.21	10	0.02	2	0.11	9	9.0	20
104	0.71	39	0.05	6	0.20	20	7.3	9
196	0.74	38	0.10	17	0.23	25	9.1	22
181	0.96	21	0.04	4	0.05	1	4.0	3
173	1.15	14	0.03	3	0.07	3	7.3	9
66	0.76	37	0.16	25	0.27	27	9.8	26
112	0.81	32	0.10	15	0.17	17	4.4	5
11	0.82	31	0.16	24	0.22	24	5.5	7
189	0.65	40	0.16	26	0.39	35	8.3	16

other sites (Table 9). Medingen shows high correlations with Syke and Binnen, yet still shows good correlations with the high-elevation sites. The estimated gain for selection at Medingen and planting at Paderborn, Kattenbühl, Holzminden and Lautenthal was in three cases greater than for selection at either Syke or Binnen. Medingen may be considered intermediate between the low and high-elevation sites. The least similar sites are Lautenthal and Binnen as indicated by a low correlation of clonal means and low gains from selection and planting between sites.

Table 7. — Rank correlation coefficients between stability parameters.

	b_i	S_{di}^2	ecovalence	S_{1j}
\bar{V}_i	0.69	0.27	0.20	-0.02
b_i		0.20	0.14	0.04
S_{di}^2			0.88	0.43
ecovalence				0.43

Comparison of clones and seedlings

Estimates of gains from further clonal selection have been given. Gains achieved from past clonal selection can be assessed by comparison of seedlings and clones. It is assumed that seedlings used for comparison are representative of populations from which clones were initially selected, and that these seedlings would be used as planting stock if clones were not used.

The mean ten-year height growth of seedlings was 1.81 meters as compared to a mean for clones of 2.09 meters (Table 4). This represents a gain of 15 percent from clonal selection and planting. Clones grew better than seedlings at all test sites except Kattenbühl. All differences were highly significant owing to the large number of degrees of freedom. The reason seedlings were superior at Kattenbühl is unclear. Clones and seedlings may have been treated differently at planting since survival of clones was much worse than survival of seedlings whereas at all other sites survival was about equal for clones and seedlings.

Conclusion

A statistically significant interaction in ten-year height growth exists between Norway spruce clones and their test sites in northern Germany. This interaction may be reduced by dividing the region into two planting zones. This would result in an increase in gain from clonal selection, within each planting zone, but also involves an increase in costs from having two clonal programs as opposed to one.

Characterization of sites by the method of genetic correlations indicates that most of the interaction is being generated between sites of high and low elevation. If the increase in overall gain justifies the increase in costs, stratification of planting zones should proceed based on elevation. Whether one or two planting zones are used, the best sites for testing within each zone may be identified using the method of genetic correlations, taking into account the heritabilities at the respective sites. The next step would be to identify environmental characteristics that determine a good testing site.

Other factors besides height growth may be important to decisions of stratification of planting regions. The Norway spruce clonal program has actually been divided into two zones above and below 300 meters. The two zones are based on differences in crown architecture of trees from different elevations and the relationship to snowbreak damage. Such information would not be available from the present study for many years. Other factors important to the stratification of planting zones may include damage from disease and insects, wind and acid rain.

Interactions may also be reduced by selecting stable clonal varieties within planting zones. A sufficient number of clones within a variety assures some population buffering. Individual buffering can be used by selecting clones that are stable as defined by the stability parameters $S^2_{(i)}$, ecovalence, and S_{1i} . Selection for stability within a planting zone involves no increase in cost to the program, but may involve a reduction in gain when clones excluded due to instability were superior in height. The gain from a reduction in clone \times site interaction must be compared with the loss from excluding clones with high growth potential.

Identification of specific environmental conditions involved in generating interactions can lead to identification of clones suited to specific environments. Such a fine-tuning of clones and environments would not be possible from a seed orchard approach to tree improvement. Within a clonal program, it would involve considerable record keeping and the increase in costs would need to be weighed against possible gains. Whatever approach is used to handle genotype-environment interactions, economics should be considered.

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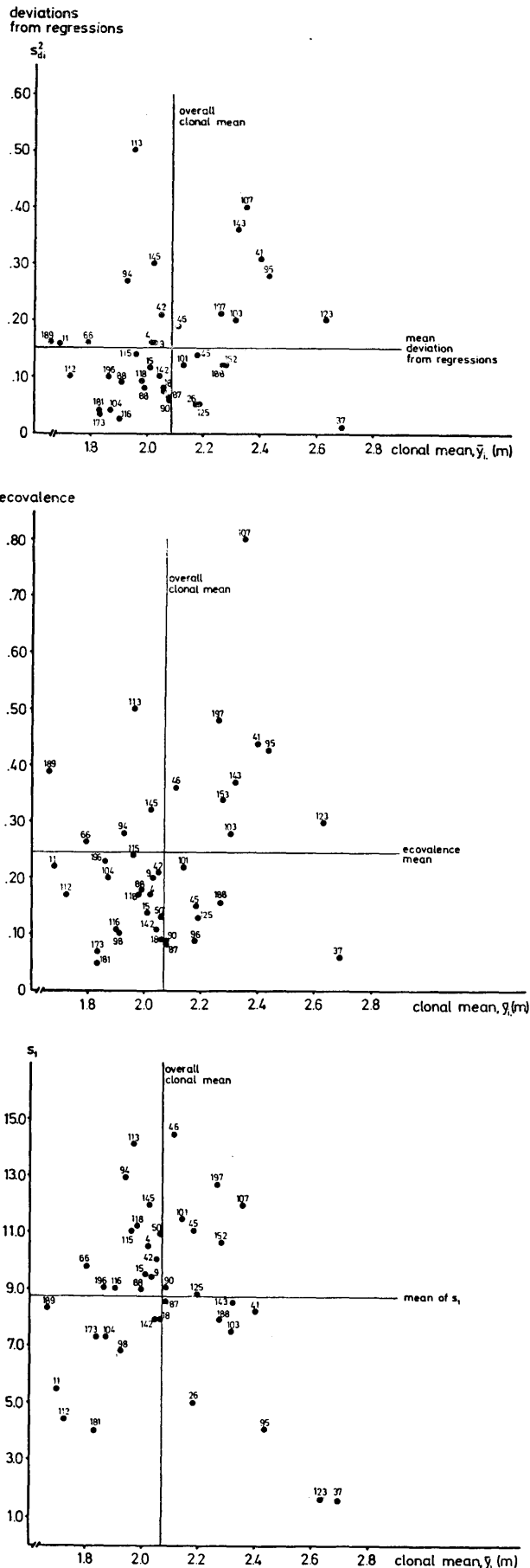


Fig. 3. — Plot of $s^2_{(i)}$, ecovalence and s_1 against clonal means.

Table 8. — Expected genetic gains from clones planted at site y after testing at site x (in meters) intensity of selection assumed to equal 1.596. Estimated clonal components of variance and repeatabilities of clonal means for each site given in margin. Gain for selection at site x with planting at all sites assumes each environment represents equal proportion of area to be planted.

Planting site y	Testing site x							overall selection	0 ² _{cy}
	Syke	Medingen	Binnen	Paderborn	Kattenbühl	Holzminden	Lautenthal		
Syke	0.490	0.382	0.339	0.252	0.286	0.269	0.188	0.439	0.112
Medingen	0.354	0.431	0.288	0.274	0.258	0.259	0.220	0.405	0.091
Binnen	0.380	0.350	0.537	0.255	0.293	0.309	0.192	0.464	0.138
Paderborn	0.166	0.195	0.149	0.253	0.164	0.168	0.131	0.228	0.038
Kattenbühl	0.197	0.192	0.179	0.171	0.280	0.180	0.168	0.256	0.044
Holzminden	0.152	0.158	0.155	0.144	0.148	0.256	0.155	0.213	0.033
Lautenthal	0.092	0.117	0.085	0.098	0.120	0.136	0.221	0.151	0.025
Overall planting	0.262	0.261	0.247	0.207	0.221	0.225	0.182	0.329	0.048
R _{cx}	0.77	0.80	0.82	0.66	0.70	0.78	0.84	0.89	

Table 9. — Correlation coefficients between clonal means for ten-year height growth among sites.

	Medingen	Binnen	Paderborn	Kattenbühl	Holzminden	Lautenthal
Syke	0.80	0.70	0.58	0.64	0.57	0.40
Medingen		0.66	0.70	0.64	0.61	0.52
Binnen			0.53	0.59	0.59	0.37
Paderborn				0.63	0.61	0.48
Kattenbühl					0.61	0.57
Holzminden						0.61

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Isolierung und Elektrofusion von Koniferenprotoplasten

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Zusammenfassung

Beschrieben wird die Isolierung von Protoplasten aus Fichte (*Picea abies* (L.) KARST.) und Kiefer (*Pinus sylvestris* L.). Deutliche Beziehungen bestehen zwischen Protoplastenausbeute, Lebensfähigkeit der Protoplasten und dem Verholzungsgrad der Kotyledonen in den verschiedenen Stadien ihrer Entwicklung. Die Stadien 7 bis 9 erwiesen sich als die geeignetsten zur Protoplastenisolierung. Ab Stadium 10 nehmen Protoplastenausbeute und Lebensfähigkeit aufgrund fortschreitender Verholzung des Gewebes signifikant ab.

Die Fusion der Protoplasten erfolgte durch elektrischen Feldimpuls nach dielektrophoretischer Sammlung der Protoplasten. Erfolgreich fusioniert wurden Protoplasten von *Picea abies* × *Picea abies*, von *Pinus sylvestris* × *Pinus sylvestris* und von *Picea abies* × *Pinus sylvestris*.

Beträchtliche Unterschiede ergeben sich hinsichtlich der Isolierungs- und Fusionsbedingungen von Koniferenprotoplasten gegenüber Protoplasten aus krautigen Pflanzen.

Schlagwörter: Koniferen, Protoplasten, Fusion.

Summary

The isolation of protoplasts of Norway spruce (*Picea abies* (L.) KARST.) and Scotch pine (*Pinus sylvestris* L.) is described. Distinct differences exist between protoplast yield, viability of the protoplasts and lignification degree of the cotyledons in their different developmental stages. The stages 7 to 9 turned out to be the most suitable for protoplast isolation. Due to progressive lignification of the tissue protoplast yield and viability decrease significantly beginning with stage 10.

Fusion of the protoplasts was effected by electrical break down after dielectrophoretic collection of the protoplasts. Protoplasts of *Picea abies* × *Picea abies*, *Pinus sylvestris* × *Pinus sylvestris* and *Picea abies* × *Pinus sylvestris* were successfully fused. Considerable difference are observed with regard to the isolation and fusion conditions of conifer protoplasts as compared with protoplasts of herbaceous species.

Key words: conifers, protoplasts, fusion.

Einleitung

In der Forstpflanzenzüchtung werden ebenso wie in Landwirtschaft und Gartenbau seit einigen Jahren mit der Anwendung von Gewebe- und Zellkulturen, speziell der Protoplastentechnik, neue Wege beschritten.

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Aus Geweben von 22 Gehölzpflanzen wurden bisher Protoplasten isoliert, bei 11 Arten ist deren Kultur gelungen. Jedoch ist außer bei *Citrus*-Arten noch keinerlei Pflanzenregeneration bekannt geworden. Auch Protoplastenfusionen sind nur von vier Arten beschrieben (АНУИА, 1984).

Demgegenüber steht die Isolierung von Protoplasten aus krautigen Pflanzen von über 500 Pflanzenarten insgesamt, von denen fast ausnahmslos alle kultivierbar sind. Belege über eine erfolgreiche Pflanzenregeneration existieren von mehr als 60 Pflanzenarten.

Diese Unterschiede deuten bereits auf die Schwierigkeiten hin, die sich aus der Arbeit mit Gehölzpflanzen ergeben. Darin liegt sicherlich auch die Hauptursache, daß sich nur ein Drittel der Ergebnisse auf Koniferen beziehen und gelungene Fusionen von Koniferenprotoplasten bisher noch nicht beschrieben worden sind.

Nachfolgend wird die Isolierung von Protoplasten aus Kotyledonen von *Picea abies* (L.) KARST. und *Pinus sylvestris* L. dargestellt und deren inner- und zwischenartliche Fusion durch die elektrische Feldimpulstechnik.

Die Versuche haben das Ziel, neue Wege und Methoden für die Forstpflanzenzüchtung, insbesondere Grundlagen Erkenntnisse zur Protoplastenfusion über Artgrenzen hinweg zu gewinnen und biotechnologische Nutzungsmöglichkeiten zu erkunden.

Material und Methoden

Anzucht der Keimlinge

Saatgut von Fichte und Kiefer wurde nach 24stündigem Einquellen in Wasser in Quarzsand ausgelegt und in einer Phytokammer angezogen (Photoperiode 14 h, 20° C, 60% rel. Luftfeuchte, 7000 lx; Dunkelperiode 10 h, 15° C, 95% rel. Luftfeuchte). Die Einteilung der Keimlinge nach Entwicklungsstadien erfolgte nach TESCHE und ZENTSCH (1978).

Die Fichtenkeimlinge konnten analog klassifiziert werden.

Protoplastenisolierung

Die Protoplastenisolierung erfolgte in Anlehnung an die Methode von DAVID und DAVID (1979).

Die Kotyledonen der Keimlinge wurden abgetrennt und in wenigen Tropfen 0,7 M Mannitlösung mit einem Skalpell längsgeteilt. Daran schloß sich die Übertragung des Gewebes in ein Enzymgemisch bestehend aus Cellulase R-10, 1 %ig, (Kinki Yakult, Nishinomiya, Japan) Rapidase 0,5 %ig, (Firma Seclin, Frankreich) und Pectinol D 0,5 %ig, (Firma Röhm, Darmstadt, BRD)