rules in Sweden, the acceptance of cutting propagated clones for commercial use is determined by the total gain compared to recommended seedling material without separating the genetic gain from other components. This means that the total gain when using mixtures of accepted clones includes effects of the cutting propagation method as well as genetic selection effects. There is reason to believe that the propagation effect is variable, which makes it difficult to generalize single quantifications of this effect.

Practical implications

A considerable part of the superiority of cuttings compared to seedlings might be caused by physiological and morphological differences for the two plant materials; i.e. the effect of the cutting propagation method has not been quantified. A suggested method for determining the genetic gain by using selected cuttings would be to compare with randomly selected bulk propagated cuttings.

Height growth comparisons in clonal tests might be improved by measuring the length of a fixed number of internodes (distance between branch whorls) from about 2.5 m height. The method has been developed to estimate site index (HÄGGLUND, 1976) and makes it possible to get good estimations of future growth.

As the establishment success plays an important role in the economy of a forest stand, it is necessary to include this effect in a selection procedure. Development of a selection index where establishment and growth capacity are combined, but considered as separate properties, might be a good solution.

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Serial Propagation in Norway Spruce (Picea abies (L.) Karst.): Results from Later Propagation Cycles

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Abstract

Maturation state can adversely affect the success of vegetative propagation, insofar as more mature material is difficult to root and tends to grow plagiotropically. One method used to retard maturation of trees in clonal tree improvement programs is serial vegetative propagation, which has the advantage of being practical for large-scale operations. Norway spruce ramets first rooted in 1968 have been re-rooted seven times, while new clones have been added regularly. Growth traits such as height, root collar diameter and fresh and dry weight, as well as form traits such as habit, tropism, root development and number of branches are compared on four trees of each three-year-old clone to evaluate the success of serial propagation in retarding maturation. There are distinct differences in the performance of seedlings and cuttings. Cuttings are generally superior in total dry matter production, even in higher propagation phases where a decrease in height can be observed. While clones that have been rooted from one to four times show fast growth and good form, some clones within later propagation phases show decreases in both traits, indicating a higher maturation state. It may be possible to select against these fast-maturing clones, thus prolonging the possible period for vegetative propagation, but a restriction of repropagation to seven or eight phases seems to be necessary.

Key words: *Picea abies*, serial vegetative propagation, juvenility, maturation.

Zusammenfassung

Bei vegetativer Vermehrung sinkt der Bewurzelungserfolg mit zunehmendem Alter und die Umstellung vom plagiotropen Wuchs dauert länger. Serienvermehrung ist eine Methode, mit der versucht wird, die Alterungseinflüsse zu verzögern. Sie hat zudem den Vorteil, daß sie gut in praktische Großvermehrung integriert werden kann. Hier wird das Wachstum (Höhe, Wurzelhalsdurchmesser, Frischgewicht und Trockengewicht) und die Form (Habitus, Tropismus, Wurzelausbildung, Zweiganzahl) von Fichtenstecklingen verglichen, die in 3jährigem Abstand ein- bis siebenmal vegetativ vermehrt worden sind, um den Einfluß der Serienvermehrung auf die Verzögerung der Alterung zu ermitteln. Bis zur vierten Vermehrungsphase zeigen die Stecklinge rasches Wachstum und gute Form. Bei höheren Vermehrungsphasen ist, klonweise unterschiedlich, ein deutlicher Alterungseinfluß festzustellen, der sich in Verringerung des Wachstums in den ersten 3 Jahren und langsamerer Umstellung in der Form niederschlägt. Durch Auslese gegen Klone, die solche Einflüsse ausgeprägter zeigen, ist es möglich, die Dauer der vegetativen Vermehrbarkeit zu verlängern. Eine Begrenzung der Serienvermehrung von Fichte auf sieben bis acht Vermehrungsphasen scheint notwendig zu sein. Zwischen Sämlingen und Stecklingen bestehen deutliche Strukturunterschiede. Die Stecklinge haben immer eine höhere Trockensubstanzproduktion. Dies gilt auch für die Vermehrungsphasen 5 bis 7.

Introductions

There are numerous advantages of vegetative propagation over seedling-based (zygotic) propagation (Libby, 1983; Libby, 1990). Vegetative propagation is independent of flowering, production can be easily tuned to the needs for planting stock, and breeding is much more flexible in adaptation to specific goals. Using rooted cuttings, proven genotypes may be easily and in great quantities replicated for practical forestry purposes. Nonadditive genetic variance may be captured by reproducing genotypes identically. Genotypes may be maintained in a vegetative propagation program that are suitable for extreme sites, while others may be maintained that would be at a competitive disadvantage in traditional forestry (ie trees with a low level of male and female strobili). In addition, vegetative propagation can be used in programs to maintain genetic diversity as designated genotypes may be held in clonebanks, used for the establishment of clonal seed orchards (Burdon 1982), or used in clonal mixtures.

Vegetative propagation of Norway spruce (*Picea abies* (L.) Karst.) has been shown to be a cost-effective and practical alternative to seed orchard seed production in tree improvement programs in Germany and other countries (Kleinschmit et al., 1973; Kleinschmit, 1974; Kleinschmit and Schmidt, 1977). It is well known that within conifer genera, maturation state has a profound influence on the success of vegetative propagation. Juvenile material is able to be used for vegetative propagation to a much greater degree than material from mature trees (Schaffalitzky de Muckadell, 1959), as it exhibits a greater readiness to form adventitious roots and to resume an orthotropic growth habit.

Maturation state has a great problem with vegetative propagation since breeders began the practice (Roulund, 1975; Libby, 1983). Breeders prefer to choose plus trees at rotation age, which is too late for successful rooting of vegetative propagules within many conifer species (Bonga, 1987). The rootability of cuttings from Norway spruce drops sharply after 10 years (Roulund, 1975), a trait seen in other conifer genera as well. When rooting of older material is successful, there is often a tendency towards presistent plagiotrophic growth (Libby, 1983). According to Fortanier and Jonkers (1976), ageing in plants has three aspects, which are chronological, ontogenetical and physiological. Ontogenetic ageing or maturation is a genetically programmed process of phase changes. Maturation has morphological, physiological, biochemical and genetic consequences. These aspects have differing weights in plant species. In most conifers, a strong irreversible genetic component (differential gene activity) is involved (Greenwood, 1987; Meier-Dinkel and Kleinschmit, 1989).

Methods developed that slow but do not halt maturation include cultural and chemical treatments of the donor plant, repeated regrafting, tissue culture, hedging and serial propagation (St. Clair et al., 1985; Pierik, 1990). Hedging is an option followed in programs with Norway spruce in Scandinavia. Complete rejuvenation is only attained during meiosis, but there is evidence to show that flower tissue can be used to induce somatic embryogenesis in hardwoods (Jörgensen, 1989). Through micropropagation and subsequent cryopreservation of somatic embryos, genotypes may be stored in a juvenile state for future breeding needs when testing results are available for the same clones (Jörgensen, 1990). However, such techniques are prohibitively expensive for full-scale practice.

Therefore research has been aimed towards finding the best way to maintain juvenile traits, particularly rooting capability, in candidate trees while information is being gathered about their quality as crop trees. Information about growth behavior of clones usually requires testing for at least ten years, during which period the maturation state of the original donor plants is increasing. The method of serial propagation of Norway spruce has been used at the Lower Saxony Forest Research Institute since 1968 (Kleinschmit et al., 1973). Since that time, new ramets from each clone in the program have been rooted every three years; the oldest clones have now passed through seven propagation cycles. Trends in characteristics of economic value or that indicate maturation state are noted.

Materials

The trees used in this study are part of the Norway spruce improvement program of the Lower Saxony Forest Research Institute. Details of the plant materials and the methods used have been described earlier in Kleinschmit et al. (1973), Kleinschmit (1974), and Kleinschmit and Schmidt (1978).

Ramets taken from four-year-old seedlings were rooted in 1968. In 1971, ramets were taken from these chronologically three-year-old cuttings and again rooted, while new ramets were taken from other seedlings. This cycle was repeated every three years, using the same provenance of spruce in all cases except for the clones in the first group of cuttings rooted in 1968. These originate from the regular selection program and were reselected for height growth after the nursery period (at age three). At the present time, the original cuttings rooted in 1968 have passed through seven propagation cycles, the cuttings rooted for the first time in 1971 through six propagation cycles, etc. (*Fig. 1*). Clones up to and including the sixth

propagation cycle are randomly selected from one provenance.

For simplification of terms, referring to a group of clones as "propagation phase one" indicates that they are cuttings from seedlings, while "propagation phase two" indicates that they are cuttings from cuttings from seedlings, and so forth. Within each propagation phase, a sample of twenty clones were selected at random. Each clone was represented by four three-year-old trees. In addition, sixteen seedlings acted as a control. These trees were destructively sampled in the winter of 1990, and characteristics were measured that were of interest both to indicate maturation state and to evaluate their quality as improved planting stock. These traits included rooting percentage, height, root collar diameter, number of first- and second-order branches, habit, tropism, form, root characteristics, fresh weight of roots, stems and branches, and dry weight of roots, stems, branches and needles.

Methods

The experimental design was completely hierarchical, with trees nested within clones nested within propagation phases. The trees were grown in completely randomized blocks. Initially, one-way, hierarchical analysis of variance was calculated using SAS (SAS Institute, 1985) (*Table 1*). Variables with nonhomogenous variances were analyzed using the Kruskal-Wallis test. Variance components were also calculated to assign the percentage of the total variance attributable to differences between propagation phases. It was considered to be more important to avoid Type II errors than Type I errors, so when significant differences between propagations phases where found, TUKEY'S studentized range test was used rather than DUNCAN'S multiple range test to differentiate between means.

The variables with nonhomogenous variance were those form traits scored by visual assessment. Habit is a measure of the symmetry of the tree when it is viewed from above. Trees were visually scored, with lower scores indicating branches and buds in all directions and higher scores indicating a tendency towards bilateral symmetry (*Fig. 2*). Tropism is a measure of the degree of non-vertical growth. Trees were visually scored, with higher scores indicating plagiotropic growth habit (*Fig. 2*). Form is an overall, subjective assessment of the shape ot the plant (*Fig. 2*). Three separate visual assessments were carried out to establish the degree of symmetry and development of the root system. Initially, the radial symmetry of the main roots was examined, with a low score given to trees with roots in all directions and a high score given to those with roots on only one side. A similar assessment was made for the radial symmetry of the fine roots. Lastly the overall state of development in relation to the stem was made, with low scores assigned to trees with well-developed root systems and high scores given to those with a poorly developed root system.

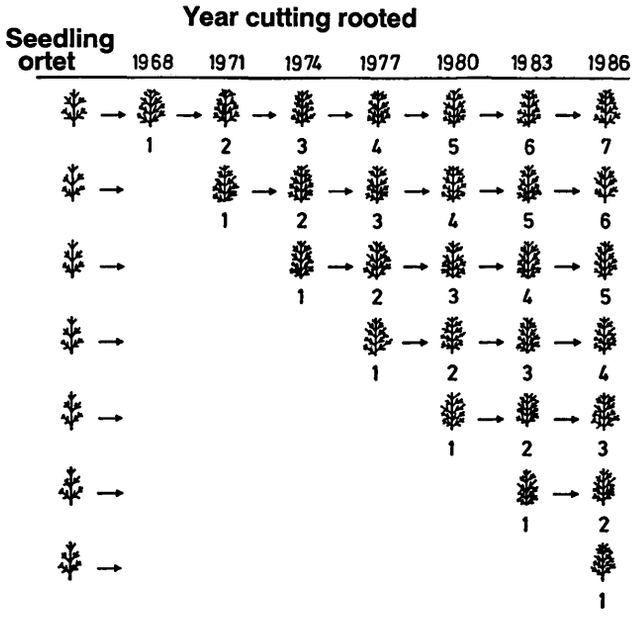


Figure 1. — Serial propagation of study material. Four-year-old seedlings were used as ortets. Ramets were grown three years before repropagating. The number below each cutting indicates the number of propagation phases. Cuttings used in this study were rooted in 1986 and destructively sampled in the winter of 1990.

Table 1. — One-way, hierarchical analysis of variance model used with components of variance.

$$Xijl = \mu + \tau i + \beta(i)j + \epsilon(ij)l$$

		Expected mean squares		
Variance source	DF	σ^2_ϵ	$\sigma^2_{KLON/ABS}$	σ^2_{ABS}
Propagation phase	6	1	4	80
Clones within phases	133	1	4	
Trees within clones	420	1		

Analysis of variance was followed by cluster and discriminant analyses using SAS. Within the SAS programs, the variables were initially standardized to a mean of zero and standard deviation of 1 to remove unit and scale effects. Classification variables, those variables that exhibited nonhomogenous variances, and variables that were the sum of other variables (ie total fresh and dry weight) were removed. Cluster analysis was performed using the procedure FASTCLUS within SAS. This method uses k-means applied to coordinate data, which is based on a least-squares criterion. Discriminant analysis was calculated using procedure DISCRIM within SAS. Following these analyses, selection was simulated by removing the bottom 10%, 20% and 30% of the clones within the last three propagations phase for the variables height and root collar diameter.

Results

I. Analyses of variance

Height

Significant differences were observed propagation phases ($p = 0.0001$), but accounted for only three percent of the total variance. A noticeable trend showed a gradual loss in height as propagation phase increased, indicating an increase in maturation state as well (*Fig. 3*). Propagation phase seven is not in full agreement with this trend. This can be explained by the selection for height applied at the conclusion of the nursery phase. The lowest values for height were shown by propagation phase six, whose

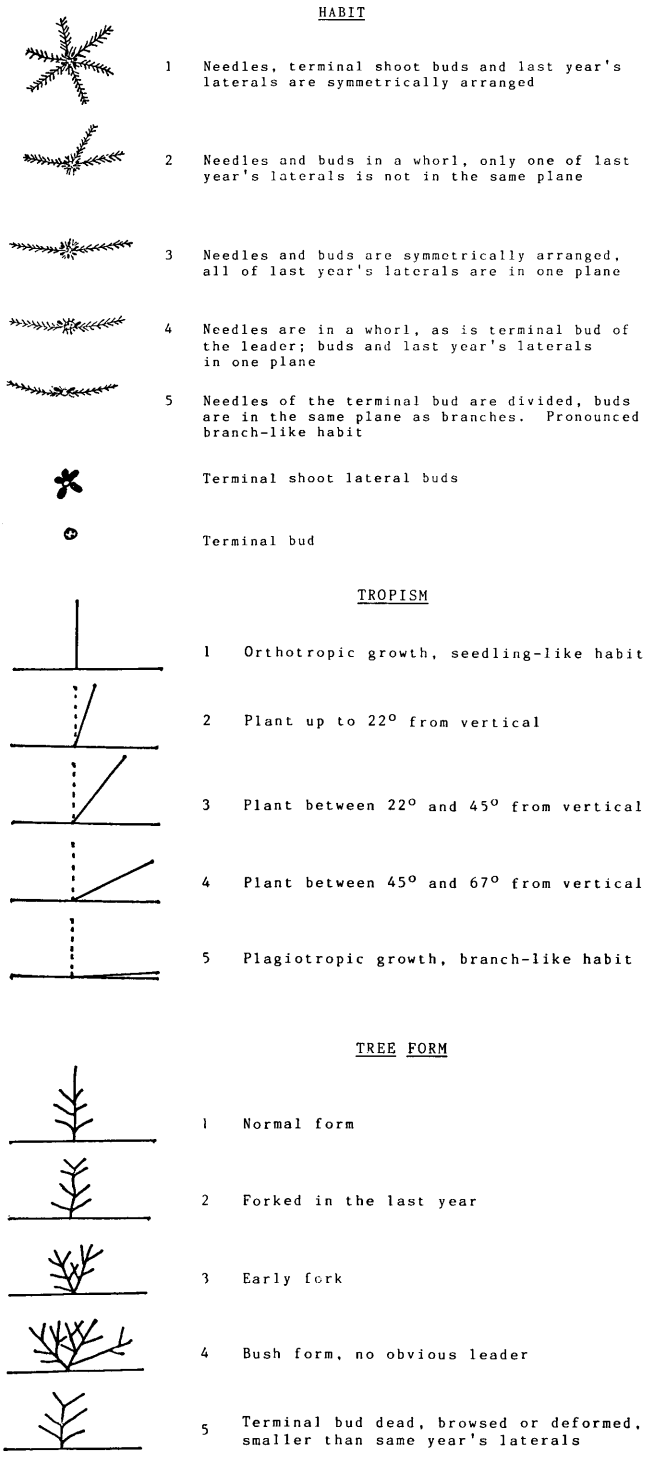


Figure 2. — Scheme for visual assessment of form characteristics in study material.

overall mean was only 91,4% of the control, and 92.3% of the overall mean. On the other hand, the tallest plants overall were from propagation phase one, with 104.5% of the overall mean and 103.4% of the mean of the control seedlings.

Root collar diameter

Significant differences were observed between propagation phases ($p = 0.02$), and accounted for one percent of the total variation. Propagation phase six was significantly smaller than propagation phase two, which had the greatest values for root collar diameter (*Fig. 4*). The trend is not as clear as it is in height growth. The two phases were 95,2% and 106.3% of the overall mean, respectively. In all cases the cuttings had a greater mean root collar diameter than the control seedlings due to the initial difference between cuttings and seedlings. Root collar diameter was correlated with fresh and dry weight ($r = 0.81$).

Habit

This variable exhibited nonhomogenous variance and was therefore analyzed using the KRUSKAL-WALLIS test, which showed significant differences between propagation phases ($p = 0.05$). However, the TUKEY test failed to show

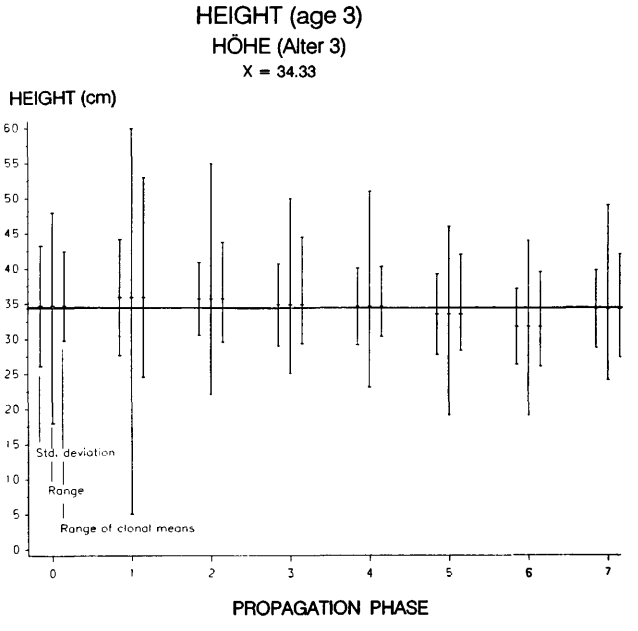


Figure 3. — Height growth at age three. Propagation phase 0 indicates the control seedlings.

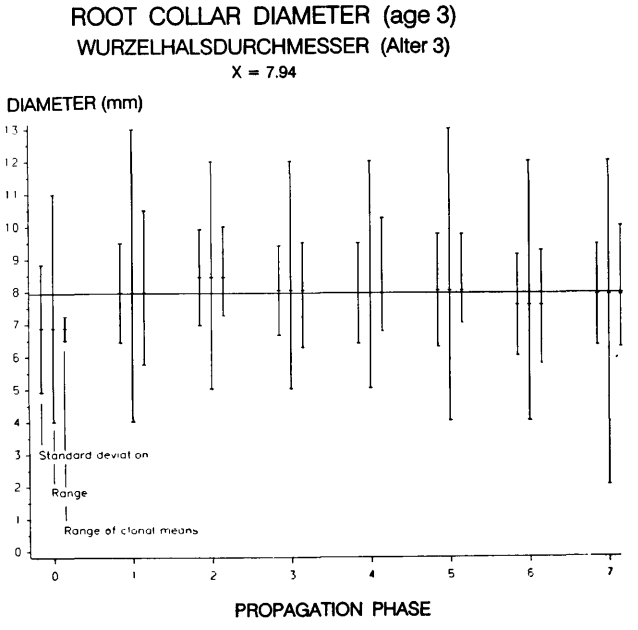
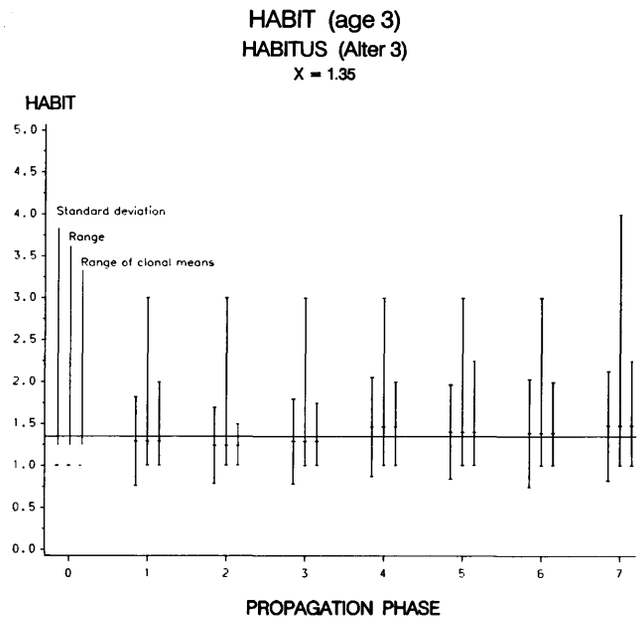


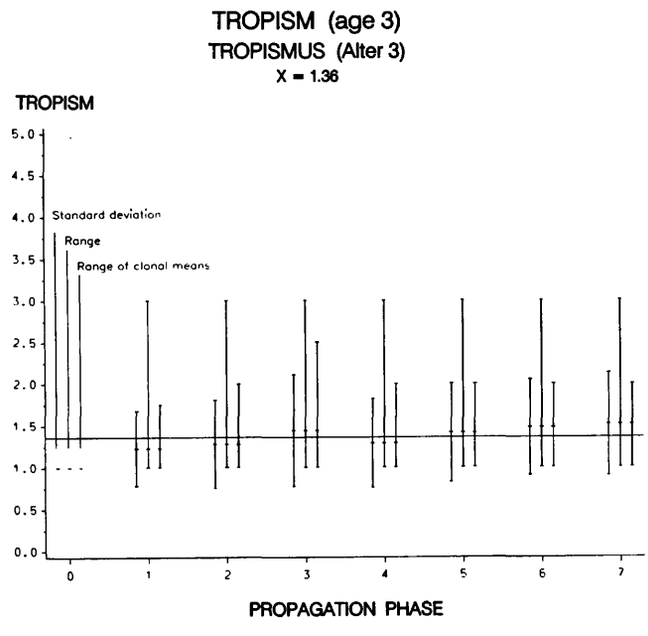
Figure 4. — Root collar diameter at age three. Propagation phase 0 indicates the control seedlings.

significant differences between any two propagation phases. A nonconsistent trend showed increasing values for habit with propagation phase (*Fig. 5*), with propagation phase seven having the highest value for habit. The control seedlings had the lowest value.

Tropism

This variable exhibited nonhomogenous variance and was therefore analyzed using the KRUSKAL-WALLIS test. It showed highly significant differences between propagation phases (p = 0.007). TUKEY's test showed that propagation phase seven had a significantly higher value then propagation phase one. The trend of further propagation phases having a higher value was more pronounced in tropism than in habit (*Fig. 5*). The most plagiotropic was propagation phase seven, which had a value 51.3% higher than the control seedlings.





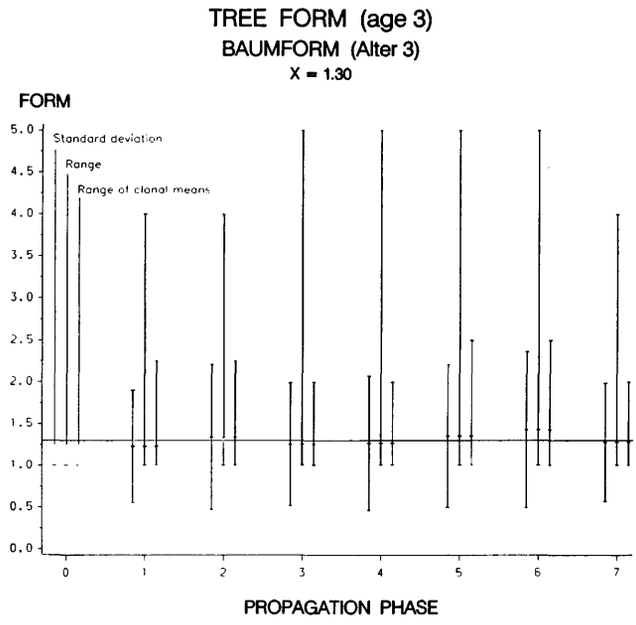


Figure 5. — Visually assessed form characteristics of study material (*Figure 2*). Propagation phase 0 indicates the control seedlings.

Tree form

This variable was also analyzed using the KRUSKAL-WALLIS test due to its nonhomogenous variance. However, no significant differences were found between propagation phases (*Fig. 5*). The TUKEY test supported this assertion.

Root assessments

No significant differences were found between propagation phases for root assessment variables.

Number of first-order branches

Significant differences were observed between propagation phases (p = 0.02), but between-phase differences accounted for only 0.3% of the total variance. Propagation phase one had the most first-order branches, while propagation phase five had the fewest. All cuttings had a mean number of fist-order branches greater than the control seedlings. This contrasts with KLEINSCHMIT and SCHMIDT (1977), where seedlings had more first-order branches than cuttings.

Number of second-order branches

There were significant differences between propagation phases (p = 0.001), which accounted for 1.7% of the total variance. Propagation phase two had significantly more branches than the latest propagation phases, with 28% more branches than propagation phase five. Once again, in all cases the vegetatively propagated trees had more second-order branches than the control seedlings. This is in agreement with KLEINSCHMIT and SCHMIDT (1977).

Fresh weight of branches

Significant differences were also discovered between propagation phases (p = 0.0001), which were responsible for 4.6% o fthe total variance. The mean branch weight of propagation phase two was 122.3% of the overall mean and 199.7% of the mean of the control seedlings. In contrast, propagation phase six had the lowest weights among the cuttings, with values of 89.1% of the overall

mean and 145.4% of the control seedling mean. The fresh weight of the branches on the cuttings was consistently much greater than on the control seedlings. There was a good correlation between fresh weight of branches and roots (r = 0.81).

Fresh weight of stem

Significant differences were found between propagation phases (p = 0.0001) which accounted for 1.0% of the total variance. The mean fresh weight of the stem in propagation phase six was only 84% of the overall mean, while in propagation phase two it was 114.3%. In addition, the mean fresh weight of stem in the control seedlings was 95.5% of the overall mean, lower than all of the means of the cutting propagation phases except phase six.

Fresh weight of roots

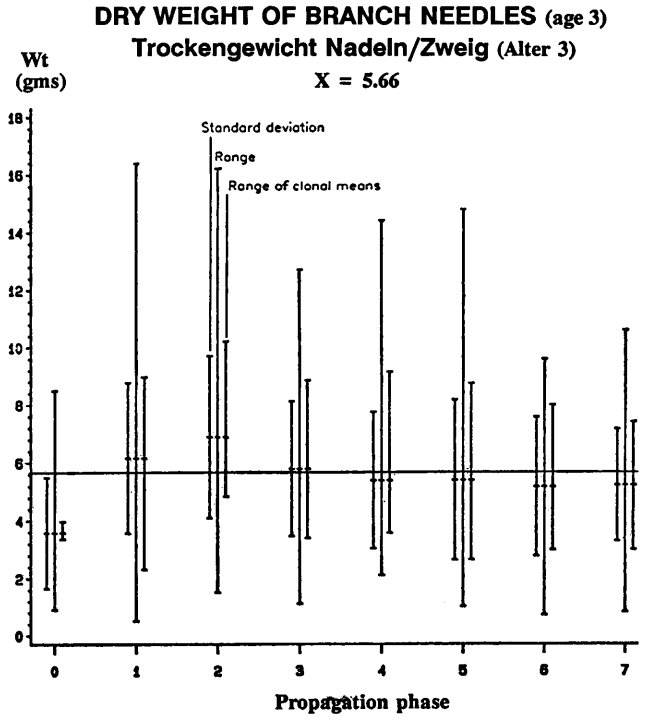
There were highly significant (p = 0.0001) differences phases (p = 0.0001), which accounted for 4.0% of the total variance. The greatest mean value was in propagation phase two, which was 119.6% of the overall mean. In contrast, the lowest value was found in the control seedlings, which was only 64% of the overall mean. Within the cuttings as a group, propagation phase six had the lowest value, which was 17% less than the overall mean. The trend is not completely consistent.

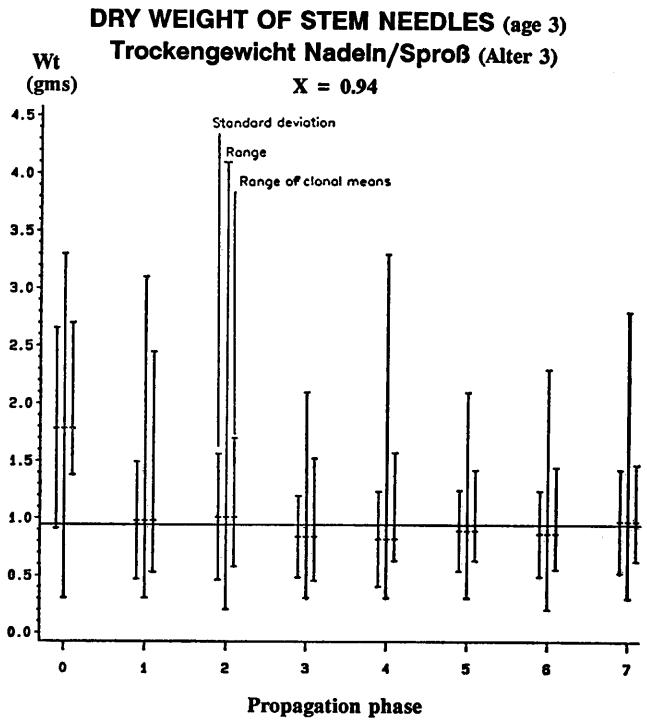
Total fresh weight

There were highly significant (p = 0.0001) differences between propagation phases, accounting for 4.6% of the total variance. The mean fresh weight of the cuttings in propagation phase two was 162.4% of the overall mean and 166.7% of the control seedlings. In contrast, the mean fresh weight of propagation phase six was 85.7% of the overall mean and 119.9% of the control seedlings. The cuttings as a group had a much larger fresh weight than the control seedlings.

Dry weight of branch needles

The differences between propagation phases were highly significant (p = 0.0001), accounting for 4.3% of the total variance. The mean dry weight of branch needles for propagation phase two was 121.8% of the overall mean, and 192.7% of the mean of the control seedlings (*Fig. 6a*). There was no propagation phase that was significantly lower than most of the others, but among the cuttings those from propagation phase six had the lowest value for this variable. The control seedlings, however, were only 63% of the overall mean.





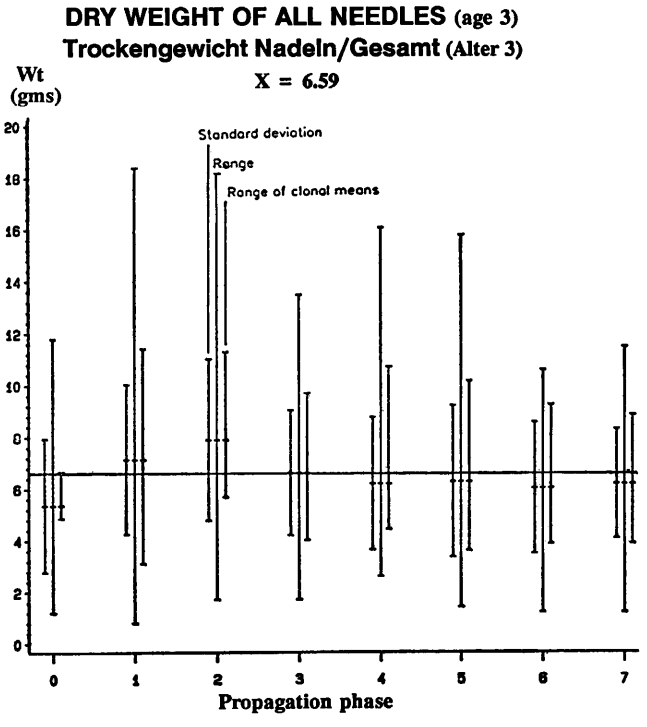


Figure 6. — Dry weight of needles at age three. Propagation phase 0 indicates the control seedlings.

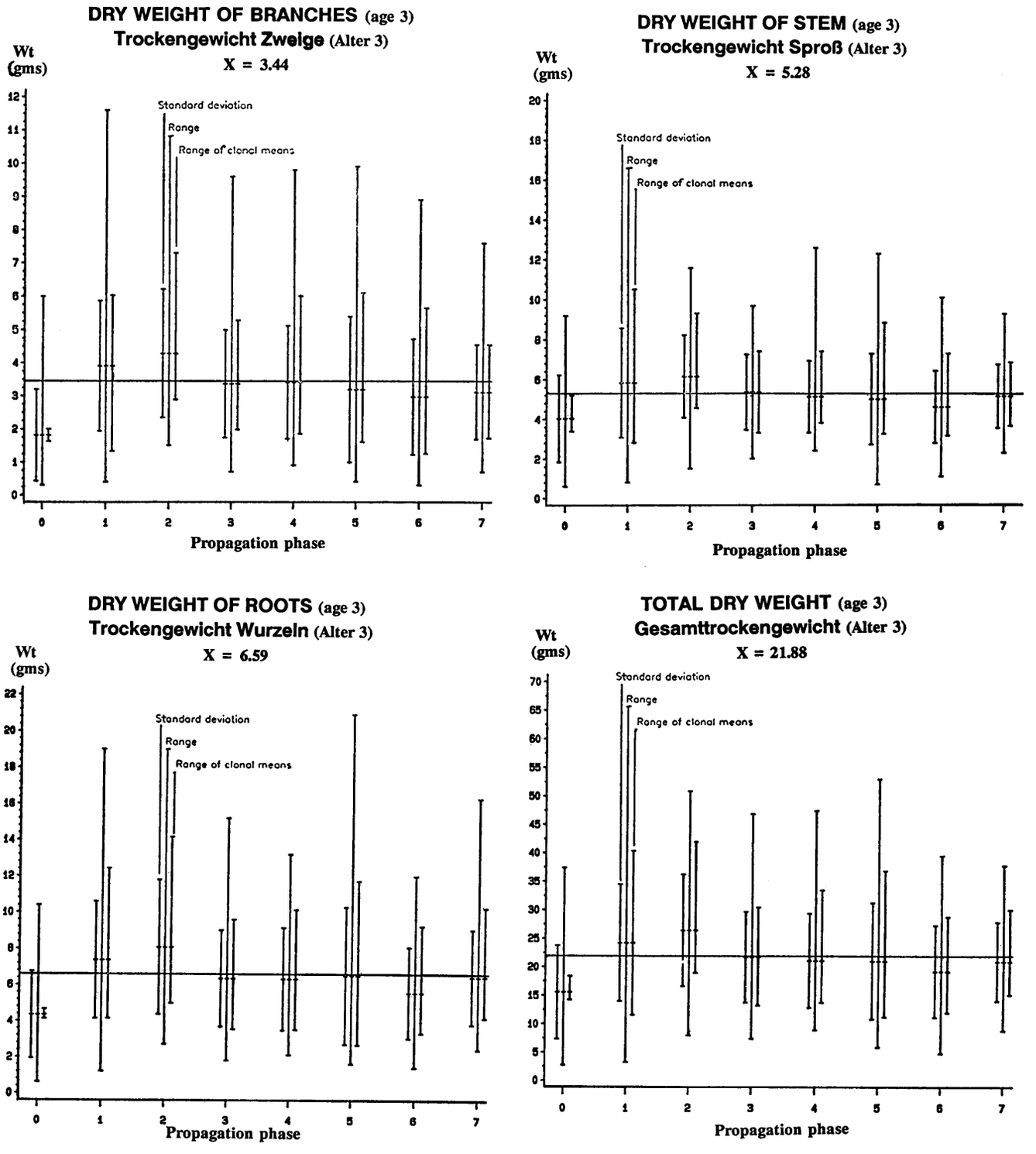


Figure 7. — Components of dry weight, and total dry weight, at age three. Propagation phase 0 indicates the control seedlings.

Dry weight of stem needles (summed)

Significant differences (p = 0.01) were found between propagation phases, accounting for 1.2% of the total variance. The control seedlings had much higher values than the cuttings, having 43.3% more weight than the mean of propagation phase two (*Fig. 6b*). Among the cuttings propagation phase two had the highest values, with 108.0% of the overall mean. The smallest values were seen in propagation phase four, the mean of which was 87.5% of the overall mean and 46.0% of the mean of the control seedlings.

Dry weight of needles (summed)

Highly significant differences were found between propagation phases (p = 0.0001), accounting for 4.5% of the total variance. The mean dry branch weight of propagation phase two was 119.8% of the overall mean, and 147.8% of the mean of the control seedlings (*Fig. 6c*). Within the

cuttings there was no propagation phase that was significantly smaller than most of the others, but again propagation phase six had the smallest values for this variable. The control seedlings, however, had a lower weight.

Dry weight of branches

Highly significant differences were found between propagation phases (p = 0.0001), accounting for 4.5% of the total variance. The mean dry branch weight of propagation phase two was 125.2% of the overall mean, while for propagation phase six it was 87.0% (*Fig. 7a*). For propagation phase two, the branch weight was 235.8% of the control seedlings, while for propagation phase six it was 163.9%. The difference between the cuttings and the control seedlings was very large for this variable.

Dry weight of stem

Highly significant differences were found between propagation phases (p = 0.0001), accounting for 4.0% of the overall variance. The mean dry stem weight of propagation phase two was 116.4% that of the overall mean, and 152.6% that of the control seedlings (*Fig.7b*). The mean dry stem weight of propagation phase six was only 87.2% that of the overall mean, and 114.3% that of the control seedlings. Again, cuttings as a group had a greater weight than seedlings.

Dry weight of roots

Highly significant differences (p = 0.0001) were found between propagation phases, accounting for 4.8% of the total variance. The mean dry weight of the roots of propagation phase two were 122.5% of the overall mean, and 186.2% of the mean of the control seedlings (*Fig. 7c*). In contrast, the mean dry weight of the roots of progagation phase six were 84.3% of the overall mean, and 128.1% of the control seedlings. Again, the weight of the cuttings was much larger than the mean root weight of the seedlings.

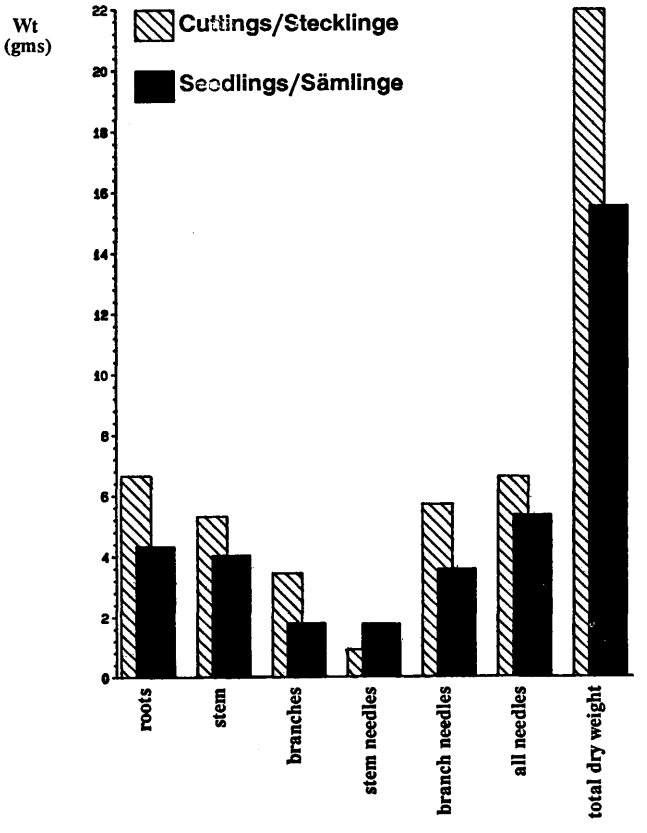


Figure 8. — Cutting/seedling comparison at age 3 for components of dry weight.

Table 2. — Distribution of individual trees from all propagation means into clusters.

Cluster	Propagation phase 1	2	3	4	5	6	7	Freq.	Nearest Cluster
1	13	19	17	17	24	19	27	136	5
2	4	7	5	9	4	0	5	34	4
3	6	8	2	2	4	0	1	23	4
4	12	16	10	11	10	13	10	82	6
5	18	8	20	19	23	27	17	132	1
6	27	22	26	22	15	21	20	153	4

Table 3. — Distribution of clonal means from all propagation phases into clusters.

Cluster	Propagation phase 1	2	3	4	5	6	7	Freq.	Nearest Cluster
1	2	4	5	2	0	1	4	18	2
2	3	6	2	1	3	3	3	21	5
3	0	1	0	2	1	3	1	8	7
4	2	0	2	0	2	5	0	11	7
5	7	6	3	7	5	3	1	32	2
6	3	2	0	1	1	0	0	7	1
7	3	1	8	7	8	5	11	43	5

Total dry weight

Highly significant differences were found between propagation phases (p = 0.0001), which accounted for 5.3% of the total variance. Overall, propagation phase two had the greatest dry biomass, and was significantly larger than any other propagation phase with the exception of propagation phase one (*Fig. 7d*). The mean total dry weight of propagation phase two was 120.7% of the overall mean. Within the cuttings as a group, propagation phase six was the smallest, with 86,6% of the overall mean. However, the cuttings as a group had a much greater dry biomass than the control seedlings. The mean total dry weight of propagation phase two as 169.9% that of the control seedlings, while for propagation phase six it was 123.4%. This is in good agreement with the findings of Kleinschmit and Schmidt (1977).

The differences between cuttings and seedlings with regards to components of dry weight are summarized in *figure 8*. In only one area, dry weight of stem needles, did the seedlings exceed the stecklings. This difference was to be seen in each propagation phase, even those later propagation phases where the mean for some characteristic was well below the overall mean.

II. Cluster analysis

If propagation phase influences act on all clones in the same direction, then clones of one propagation phase should be more similar than clones of other propagation phases. One method of testing this hypothesis is

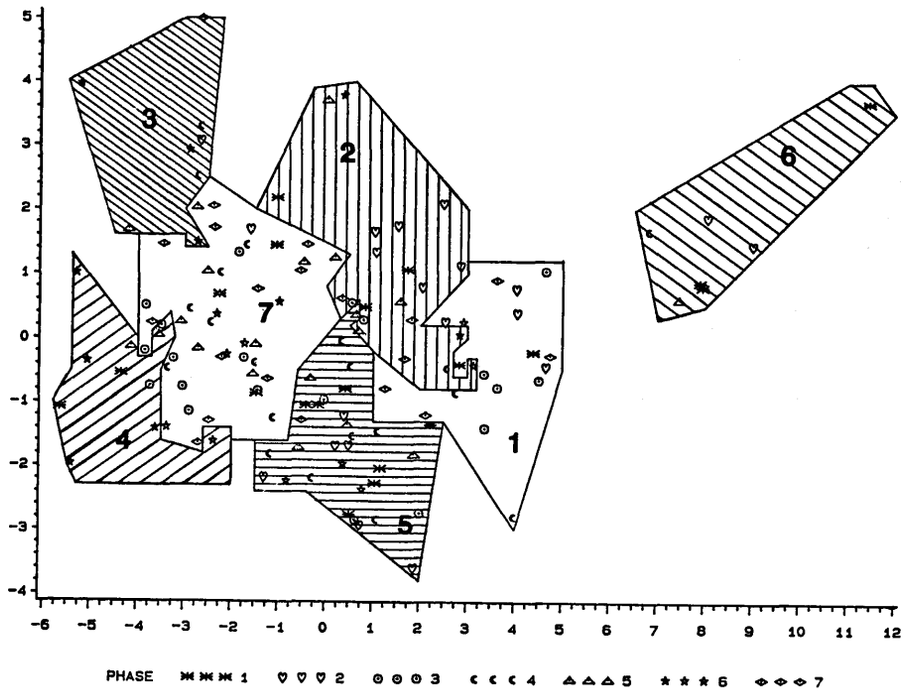


Figure 9. — Cluster distribution of clonal means. X- and Y-axes are canonical variables that summarize all the quantitative variables. Numbers correspond to cluster descriptions in *Table 3.*

cluster analysis, where approximately the same number of groups is formed as propagation phases exist. All variables evaluated in the ANOVA are used in the cluster analysis, with the exception of those that show non-homogenous variance and those variables that are non-independent as they are totals from other variables. No differentiation in variable weights was made.

Cluster analysis was initially performed with the individual-tree values of all the cuttings in the study. The control seedlings were omitted as they comprised a much

Table 4. — Redistribution of individual trees from all propagation phases by discriminant analysis. Underlined values are the number re-allocated to the same propagation phase.

	Propagation phase							
From phase	1	2	3	4	5	6	7	Total
1	<u>26</u>	11	7	11	4	10	11	80
2	13	<u>28</u>	11	11	7	7	3	80
3	8	6	<u>37</u>	5	5	15	4	80
4	11	10	12	<u>22</u>	7	14	4	80
5	4	8	14	7	<u>24</u>	17	6	80
6	7	3	12	11	8	<u>28</u>	11	80
7	16	4	13	6	16	13	<u>12</u>	80
Total	85	70	106	73	71	104	51	560
%	15.18	12.50	18.93	13.04	12.68	18.57	9.11	100.00

Error count estimates for phase:

	1	2	3	4	5	6	7	Total
Rate	0.6750	0.6500	0.5375	0.7250	0.7000	0.6500	0.8500	0.6839
Priors	0.1429	0.1429	0.1429	0.1429	0.1429	0.1429	0.1429	

Table 5. — Redistribution of clonal means from all propagation phases by discriminant analysis. Underlined values are the number re-allocated to the same propagation phase.

From phase	Propagation phase 1	2	3	4	5	6	7	Total
1	8	3	3	2	1	2	1	20
2	4	9	3	2	1	1	0	20
3	2	1	11	3	1	1	1	20
4	0	2	4	8	1	2	3	20
5	0	1	1	2	8	4	4	20
6	2	0	1	3	2	10	2	20
7	2	0	0	4	4	5	5	20
Total	18	16	23	24	18	25	16	140
Percent	12.86	11.43	16.43	17.14	12.86	17.86	11.43	100.00

Error count estimates for phase:

	1	2	3	4	5	6	7	Total
Rate	0.6000	0.5500	0.4500	0.6000	0.6000	0.5000	0.7500	0.5786
Priors	0.1429	0.1429	0.1429	0.1429	0.1429	0.1429	0.1429	

smaller group than any of the propagation phases (16 trees rather than 80). Cluster analysis was performed with a specified maximum number of seven clusters, corresponding to the seven propagation phases. Clusters of fewer than five observations were restricted. Results are summarized in *table 2.*

The results of this evaluation show no clear trend. Clones of every propagation phase are to be found in nearly every cluster, and clusters are made up of similar numbers of trees from each propagation phase.

Cluster analysis of individual trees was followed by cluster analysis using clonal means. This was done purely for the value to breeders and others interested in overall clone performance, as selection of material for breeding purposes is often based on group rather than individual performance. A maximum number of seven clusters were permitted, and clusters with less than three observations were restricted. Results are summarized in *table 3.*

The same trends hold true when looking at the results of clonal mean evaluation. All the propagation phases are represented in at least five clusters. Canonical variables that summarize many variables in one linear combination were calculated to summarize the trends over all variables within each propagation phase ,then graphed (*Fig. 9*). The graph shows a thorough distribution of propagation phases, with no obvious clusters.

III. Discriminant analysis

Cluster analysis was followed by another multivariate procedure, discriminant analysis. While clustering assumes no prior information about groups, discriminant analysis was performed using propagation phase as a classification variable. As before, individual-tree values were used initially. Results are summarized in *table 4.* Following individual-tree analysis, clonal means were again used. Results are summarized in *table 5.*

The results show clearly that in both individual- tree and clonal means analysis, the greatest number of clones per phase was reallocated to the same group. Despite this tendency, in the individual-tree analysis the highest number of trees so allocated was 37, less than 50% of the total. The lowest number so allocated was 12, or 15%, Smoothing the variation by using clonal means increased the percentage reallocated to the same group. However, in only two cases did this reach 50% of all clones, and it was little as 25% of the clones in propagation phase seven.

IV. Simulated selection

If clones do not mature at the same rate, selection against fast maturation by selecting for fast growth may be possible, thus prolonging the possible time of repropagation. Selection was simulated by removing the lowest 10%, 20% and 30% of clones from the last three propagation phases. This was done by evaluating clonal performance for height and root collar diameter, and eliminating the lowest-performance clones.

When height was used as the selection variable, improvements were obtained in most characteristics but on a lower level than with root collar diameter. There is an average increase of 1.8% in height over the last three propagation phases when the lowest 10% of clones in the last three propagation phases are dropped (*Fig. 10a*). This increases to 3.5% when the lowest 20% of clones are dropped (*Fig. 10b*) and again to 5.4% when the lowest 30% of clones are dropped (*Fig. 10c*). Increases in root collar diameter range between 0.4% and 2.2% (*Fig. 11*). The increase in fresh weight goes as high as 5.9% at the

30% selection level, while dry weight lags behind at 5.4%. Values for form traits were not substantially changed at any level of selection. The increase in weight can be seen in all categories but is most pronounced in the increase in dry weight of stem needles, 6.2% at the 30% selection level.

In addition to the quantifiable gains, a drop in the number of significant differences between propagation phases was seen (*Fig. 12*). The ANOVA and TUKEY's test showed that within the set of variables with homogenous variance, there were 65 separate between-phase significant differences. Eliminating the bottom ten percent of clones in the last three propagation phases (a total of six clones) only reduced the 65 significant differences by one. However, a 20 percent reduction in the last three propagation phases brought the number down to 55, and a 30 percent reduction brought the number to 45. Significant differences between propagation phases for height growth were eliminated, bud most of the significant differences between phases associated with fresh and dry weight remained.

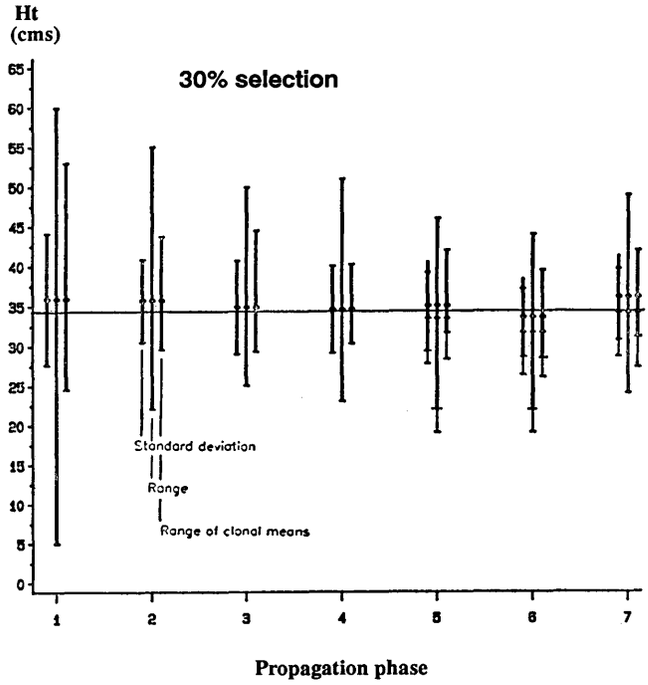
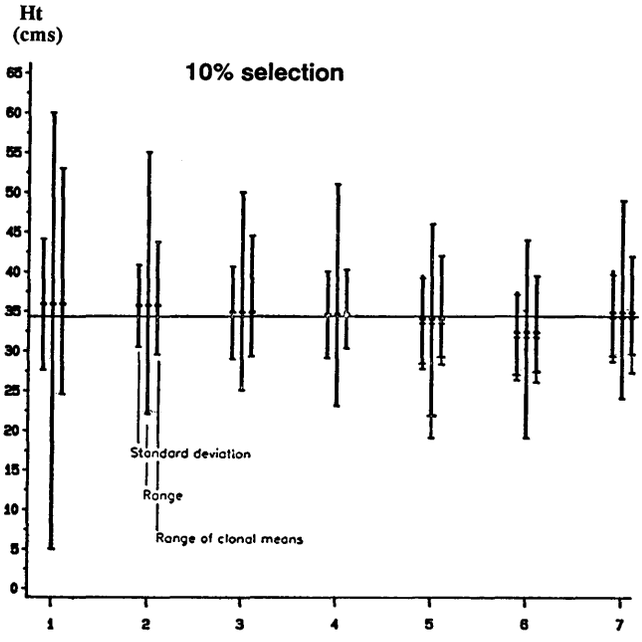
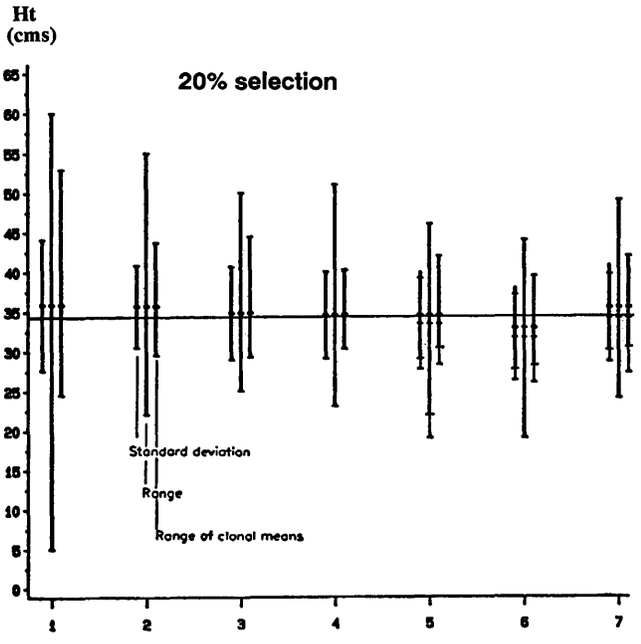
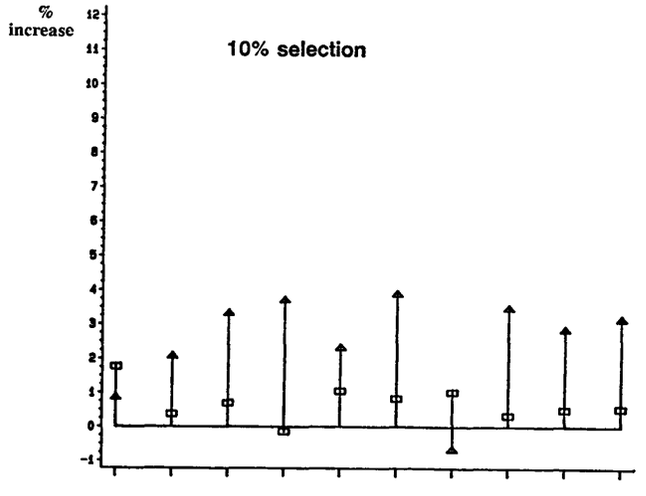


Figure 10. — Increase in height growth in the last three propagation phases after selection is simulated by the removal of the bottom 10%, 20% and 30% of clones.

Selection based on root collar diameter resulted in improvements in most characteristics (*Fig. 11*). Removal of the bottom 10% of clones yielded a 2.1% increase in root collar diameter, and removal of the bottom 20% or 30% of clones increased the percent improvement to 4.0% and 5.9%, respectively. Height was not greatly affected by 10% selection, showing an improvement of only 0.9%. However, dropping 20% of clones brought that to 1.6%, and a 30% removal brought the height up by 2.5%. Form traits such as habit, tropism and root system development showed either no change or a slight improvement. Fresh and dry weight experienced gains of about 3.3% per 10% of clones dropped. Within fresh and dry weight, the increase is ap-







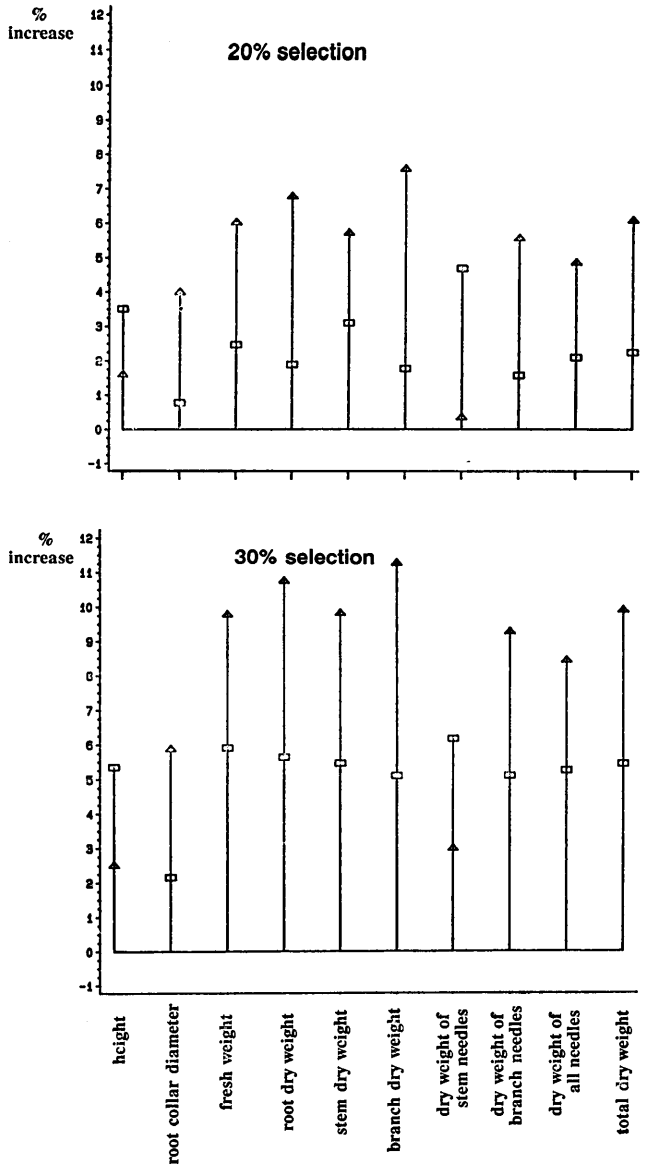


Figure 11. — Percentage increase in growth traits in the last three propagation phases after simulated selection. Triangles indicate selection by root collar diameter, squares by height.

parent in all categories — roots, stems, branches and needles — but the greatest gains are seen in dry branch weight, 11.3% at the 30% selection level.

As with selection for height growth, the elimination of the worst clones in the last three propagation phases served to reduce the total number of significant differences between phases (*Fig. 12*). The initial 65 decreased to 53 by a ten percent reduction in the number of clones in the last three phases, and to 38 when twenty percent of the clones in the last three phases were dropped. When the lowest thirty percent were dropped, the number of significant differences dropped to 24. Again, all of the significant differences between phases for height growth were lost, but also many for fresh and dry weight as well as the number of first- and second-order branches.

The efficiency of selection using either height or root collar diameter is demonstrated in *Figure 11*. It is clear that the percentage increase in most traits is somewhat higher when selection is based upon root collar diameter rather than height, with two exceptions: the dry weight of stem needles, and height growth itself. Whether it is more advantageous at the age of three to select for height or for volume has not been well established.

Discussion

The gradual loss in height and root collar diameter, as well as in other traits, shows that the later propagation cycles are retaining a more mature state and lack the fast initial growth typical of seedlings in the nursery stage. If in fact maturation is controlled by activities within the shoot apex, as is suggested by Schaffalitzky de Muckadell (1959) and Robinson and Wareing (1969), it is possible that the technique of serial propagation slows but does not arrest maturation due to the fact that in each propagation cycle the shoot apex is transferred completely to the new plant.

It can be seen, however, that the earlier propagation phases show the fast growth and form of seedlings, coupled with the greater weight gain in roots, stem and branches characteristic of the stecklings in this study. The cuttings from propagation phase two have the highest values for several variables, including the weight variables. It is possible that the act of serial propagation reinvigorates the meristem without actually rejuvenating it, at least to the level of two propagation cycles. After a limited number of propagation cycles, the increasing maturation state of the clone can be seen in its slower height growth and greater disposition towards plagiotropic growth, although the total production of dry matter

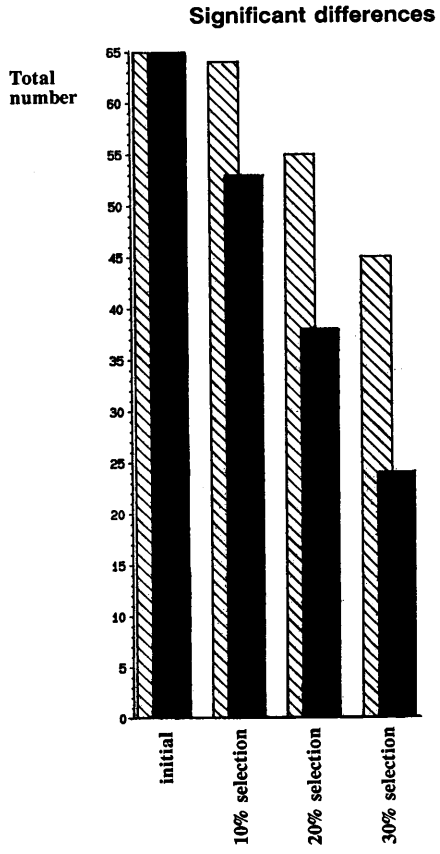


Figure 12. — Decrease in total number of significant differences between propagation phases with selection. Of 378 possible comparisons, 65 are initially significant. Slashed bars represent selection based on height growth, solid bars on root collar diameter.

is superior in all propagation phases when compared to the seedlings, as can be seen in *figure 8*. Trees within this study have always been evaluated at the conclusion of the nursery stage. It would be valuable to evaluate the trees some years afterwards as plagiotropic growth habit tends to disappear with age.

Although between-propagation phase differences are statistically significant in most cases, the make up a very small amount of the total variation. Often when the between-phase difference was significant, the difference between clones within the same propagation phase was also significant, and accounted for a greater percentage of the total variation, often on the order of three to four times as much. This would indicate that while maturation state does tend to move clones in approximately the same direction, clones respond substantially differently to its influences. Another possibility is that the differences between clones for growth in height, root collar diameter and other traits are unrelated to maturation state but are able to essentially overwhelm the effect of maturation state by their much higher variation.

That the between-phase differences are small is affirmed by the findings from the cluster analysis and the discriminant analysis. The liberal scattering of the propagation phases across the clusters as seen in *figure 9* and *tables 2* and *3* show that many clones have more in common with clones in other propagation phases than with clones in the same propagation phase. The considerable redistribution within the discriminant analysis shows this as well in *tables 4* and *5*.

The considerable clonal variation has practical implications, among which is that selection against poorly performing clones can level out propagation phase influences for quantitative traits. This is proven by the clones of propagation phase seven, where selection for fast growth took place at an early age, as well as by results of the theoretical selection study. The simulated selection showed that the number of significant between-phase differences could be reduced by selecting against the slowest-growing clones in the last three propagation phases. Using the data sets generated for the simulated selection experiments, ANOVAs showed that the amount of variation attributable to between-phase differences was reduced in almost every case, and TUKEY's tests showed that the total number of significant between-phase differences could be substantially reduced by selection. *Figure 12* illustrates the decrease in the number of significant differences seen when the poorest clones are dropped from the last three propagation phases. There are 378 possible comparisons between propagation phases for all the normally distributed variables. While the overall data set has sixty-five significant differences, this number may be greatly reduced by selection.

For breeding programmes using vegetative propagation, the unfortunate conclusion of this study is that serial propagation is not able to stop maturation completely. Even if rooting percentage does not decrease, vigour and growth habit of the clones at the end of the nursery stage is affected increasingly with increasing propagation phase. Since clones show considerable variation, selection against these negative trends can diminish these negative effects to a certain extent. It seems however necessary to restrict serial propagation to a maximum of seven or eight phases. From a breeding point of view, this is not necessarily a disadvantage, since Norway spruce starts flowering at an age of 20 years and the best clones can be reintroduced into a generative cycle at this stage of development. It limits the overrepresentation of certain clones in practical forestry at the same time. The disadvantage is that superior clones, once identified at an age of 10 to 15 years, can only be repropagated for another 10 years. This restricts possible genetic gains. This seems to be inevitable until rejuvenation techniques for fully mature plant material are developed for spruce.

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