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Multiclonal Mixtures and Number of Clones

I. Number of Clones and Yield Stability (Deterministic Approach without Competition)

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Summary

Theoretical investigations on the necessary number of clones in clonal mixtures have been performed according to phenotypic yield stability. For each clone i, $i = 1,2,..,n$, a parameter u_i with $0 \leq u_i \leq 1$ has been introduced reflecting the different survival-rates of the clones (with respect to the entire rotation time). The yield of a clonal mixture can be expressed quantitatively by using the two characters 'survival-rate' and 'yielding-ability'.

Applying several simplifying assumptions (proportionality between 'survival-rate' and 'yielding-ability'; non-consideration of the competitive effects between the clones in the clonal mixture; equal proportions of the n clones in the initial composition of the clonal mixture etc.) a very simplified and purely deterministic approach has been used in this first paper: Equally spaced u_i's, $i = 1,2,..,n$, between the lowest and the largest u_i-value (Min u_i = k and $i = 1,2,..,n$ Max $u_i = 1$). The total yield μ of the clonal mixture can be $i = 1,2,..,n$ explicitly expressed as a function of k and n. All further studies and conclusions proceed from this $\mu = \mu(k,n)$.

For different conditions and procedures, for example:

1. $|\mu(n) - \lim \mu(n)|$ lower than g_1% of this limit or
2. | range of $\mu(k)$ — maximal range| lower than g_2 % of this maximal range or
3. realization of a certain, given yield-difference, which results from a certain required increase of the number of clones

numerical results on necessary clone numbers can be calculated.

As a very rough and only the numerical magnitude characterizing statement these results can be summarized as follows: The necessary clone numbers are in the tens rather than just a few clones or in the hundreds.

Finally, the possible restrictions of the numerical results, which may be caused by the different simplifying assumptions have been discussed critically. It can be shown, that for practical applications these simplifications are of no serious or biasing effect on the numerical magnitude of the calculated necessary numbers of clones in clonal mixtures.

Key words: Clonal mixtures, number of clones, yield stability, deterministic approach without competition.

Zusammenfassung

Bezüglich des Aspektes „phänotypische Stabilität" werden theoretische Untersuchungen zur Frage der notwendigen Anzahl von Klonen in Klonmischungen durchgeführt. Für jeden Klon i, $i = 1,2,..,n$, beschreibt ein Parameter u_i mit $0 \leq u_i \leq 1$ die unterschiedlichen Überlebensraten der Klone (bezogen auf die gesamte Umtriebszeit). Die Leistung einer Klonmischung läßt sich durch Heranziehung der beiden Merkmale „Überlebensrate" und „Ertragsfähigkeit" quantitativ angeben.

Unter einer Reihe von vereinfachenden Annahmen (Proportionalität zwischen „Überlebensrate" und „Ertragsfähigkeit"; Nichtberücksichtigung von Konkurrenzeffekten zwischen den Klonen in der Klonmischung; gleiche Anteile der n Klone in der Anfangszusammensetzung der Klonmischung u. a.) wird hier zunächst ein sehr vereinfachter, rein deterministischer Ansatz gewählt: Gleichmäßige Verteilung der u_i, $i = 1,2,..,n$, zwischen dem kleinsten und dem größten u_i-Wert (Min u_i = k und Max $u_i = 1$). Der Gesamtertrag μ der Klonmischung kann dann sehr einfach als Funktion von k und n explizit ausgedrückt und genauer untersucht werden.

$i = 1,2,..,n$ $\quad$ $i = 1,2,..,n$

Für unterschiedliche Bedingungen und Vorgehensweisen, wie z. B.:

1. $|\mu(n) - \lim \mu(n)|$ kleiner als g_1% von diesem Grenzwert oder
2. | Variationsbreite von $\mu(k)$ — maximale Variationsbreite | kleiner als g_2% von dieser maximalen Variationsbreite oder
3. Forderung eines bestimmten Leistungsunterschiedes, der bei einer bestimmten, geforderten Erhöhung der Klonanzahl auftritt

lassen sich konkrete numerische Angaben über erforderliche Klonanzahlen ableiten.

Als sehr grobe, nur größenordnungsmäßige Angabe kann man diese Resultate dahingehend zusammenfassen, daß die erforderlichen Klonanzahlen eher in 10er Größenordnungen, als bei einigen wenigen Klonen oder bei 100er Größenordnungen liegen.

Abschließend werden die durch die vereinfachenden Annahmen bedingten möglichen Einschränkungen der Ergebnisse kritisch diskutiert. Es zeigt sich, daß für praktische Anwendungen diese Vereinfachungen keinen allzu großen Einfluß auf die numerische Größenordnung der erhaltenen notwendigen Klonanzahlen haben.

Introduction and Problem

Clonal plantations are of an still increasing interest and importance in breeding programs and for commercial use. One reason for this remarkable tendency may be the fast progress in vegetative propagation, for example by using cuttings and tissue culture techniques. For very different tree species clones can be produced in an extent relevant for practical forestry. The negative experiences with genetically uniform varieties in agricultural crop science are well-known to forest tree breeders. Therefore, most of them propose the development of multiclonal varieties to maintain some genetic diversity in the populations. High production with minimal risk are the uncontested criteria and requirements.

The main problem of this series of publications 'evaluation of necessary numbers of clones in clonal mixtures' has been intensively discussed in the last years. An extensive literature review has been given by HUEHN (1984).

Numerous proposals concerning the necessary number of clones have been presented in the literature. They range from very vague to very definite statements with numerical recommendations from a few up to several hundred or even several thousand clones per clonal mixture (for example:

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KLEINSCHMIT, 1973; KLEINSCHMIT, 1974; TIGERSTEDT, 1974; LINDGREN, 1977; KLEINSCHMIT, 1979; HEYBROEK, 1981; NANSON, 1982; HALL, 1982; LIBBY, 1982; BURDON, 1982; HEYBROEK and VAN TOL, 1982; KLEINSCHMIT, 1983). Most of these recommendations have been motivated by practical reasons and experiences and/or theoretical considerations. But these theoretical results have not been derived from adequate statistical and quantitative-genetic models and approaches. Only a few of such theoretical studies are known using mathematical and statistical procedures (MARSHALL and BROWN, 1973; MARSHALL and ALLARD, 1974; TRENBATH, 1977; KAMPMEIJER and ZADOKS, 1977; LIBBY, 1982; KRUSCHE, 1982; OSTERGAARD, 1983). But these studies are mainly concerned with special aspects like: development of epidemics, analysis of interactive effects in mixtures etc. No general theoretical approach of evaluating necessary numbers of clones in clonal mixtures has been worked out.

In a series of papers theoretical results on the necessary number of clones in clonal mixtures shall be published with respect to different relevant aspects of this problem (yield stability; yielding ability; juvenile- mature correlations; risk considerations).

In these investigations we don't consider successive generations. Only one period from the initial composition of the clonal mixture until the final harvest shall be analysed.

Because of the extremely complex dependencies between a large number of influencing factors and the lack of knowledge on their interactions and even on their main effects theoretical results can be only derived by assuming several simplifying assumptions. The possible biasing effects of these simplifications must be critically considered and discussed when summarizing and extrapolating the theoretical results to a recommendation of necessary numbers of clones in clonal mixtures (HUEHN, 1984).

In most cases an increasing empirical knowledge — for example, clone-specific resistance properties, genotype-environment interactions etc. — leads to a reduction or specification of this series of assumptions. Therefore, the underlying models and assumptions for the following theoretical studies must be formulated in such a general way and, of course, in accordance to the relevant empirical facts that they include all possible practical situations. All further precisions of the assumptions lead to a more exact determination and confinement of the clone numbers. But the rough numerical magnitude of these necessary clone numbers must remain nearly unchanged under these generalizations and improvements (HUEHN, 1984).

The aim of this first paper is to give some quantitative approaches and numerical results concerning the necessary number of clones in clonal mixtures with respect to phenotypic stability using a simple deterministic approach (without considering the competitive effects between the clones in the mixture).

Phenotypic stability will be measured by the variance-concept, that means: maximum stability = constant yield under varying conditions. These conditions may be, for example, different environments (locations, years, silvicultural practices), different compositions of the mixtures etc.

Many other stability concepts and parameters are available. But the variance-concept of stability will be the most appropriate approach for applications in silviculture with their spatial and temporal environmental heterogeneity.

Of course, this stability-concept will be most adapted to the fast growing tree species like poplars and aspen. These applications can be characterized by

1. short rotation time,
2. small, disconnected production areas,
3. reduced environmental variability and
4. decreasing genotype-environment interactions.

But the results of the following theoretical investigations are not at all limited to the fast growing tree species. These results are of an extended validity if the parameters involved are defined specifically for the different tree species.

Theoretical Investigations and some Numerical Results

For each clone i, i = 1,2, . . , n, we introduce a parameter u_i with $0 \leqq u_i \leqq 1$ reflecting the different survival of the clones (u_i = relative survival parameter) with respect to the entire rotation time. The effects of all biotic and abiotic factors are summarized in these u_i. The u_i, therefore, are very global measures of the fate of the single clones during the total rotation time.

The final production of a clonal mixture can be described quantitatively by applying two characters and parameters: 'mortality' [natural and artificial (= thinning)] or survival on the one side and 'yielding-ability' on the other side:

$$\text{TOTAL YIELD} = \sum_{i=1}^{n} \left(\frac{f_i \cdot u_i}{\bar{u}} N\right) \cdot L_i \qquad (1)$$

where:

f_i = frequency of clone i in the initial composition of the mixture,

u_i = relative survival parameter of the clone i,

$\bar{u}$ = mean of the u_i's: $\bar{u} = \sum_{i=1}^{n} f_i u_i$,

n = number of clones,

L_i = mean yield of clone i (per plant) and

N = final number of plants.

The following theoretical investigations are based upon the assumption of a proportionality between L_i and u_i (see 'Discussion'):

$$L_i : L_j = u_i : u_j \quad \text{(for each i and each j)} \qquad (2)$$

Without loss of generality the maximal value of the u_i's may be assumed to be one and the corresponding L_i will be denoted by L_{max}. Using this clone as a reference clone one obtains:

$$L_i : L_{max} = u_i : u_{max} = u_i : 1 \quad \text{or}$$

$$L_i = \frac{u_i L_{max}}{u_{max}} = u_i L_{max} \quad \text{(for each i)} \qquad (3)$$

From (1) and (3) it follows:

$$\text{TOTAL YIELD} = \frac{NL_{max}}{u_{max}} \cdot \sum_{i=1}^{n} \frac{f_i u_i^2}{\bar{u}} = NL_{max} \cdot \sum_{i=1}^{n} \frac{f_i u_i^2}{u} \qquad (4)$$

The following theoretical studies will be only performed for the special case of equal proportions of the clones in the initial composition of the mixture: $f_i = \frac{1}{n}$ for each i. For practical applications this will be a realistic assumption (see: HUEHN, 1984 and 'Discussion').

For the total yield (expressed in NL_{max}-units) (4) gives:

$$\text{TOTAL YIELD} = \frac{\sum_{i=1}^{n} u_i^2}{\sum_{i=1}^{n} u_i} \qquad (5)$$

The *total yield* will be denoted by μ.

In this paper the following simple deterministic approach shall be discussed: We denote the minimal u_i, $i = 1,2,..,n$, with k and all the u_i shall be equally spaced between 1 and k (Max $u_i = 1$ and Min $u_i = k$). Low k, therefore, means a $i = 1,2,..,n$ $\quad i = 1,2,..,n$
high variability of the u_i's of the n clones, while a large numerical value of k expresses a high similarity of the u_i's of the different clones.

Of course, this assumption of an equal distribution of the u_i's in the interval from k to 1 will be a strong simplification. But the more realistic assumption of unequally distributed u_i's will not alter the rough numerical magnitude of the necessary clone numbers for clonal mixtures too much. Because we are mainly interested in this approximate numerical level the foregoing simple deterministic approach may be justified (see, 'Discussion').

For u_i, $i = 1,2,..,n$, we obtain:

$$u_i = 1 - \frac{1-k}{n-1}(i-1) \qquad (6)$$

For $i = 1$ we have $u_1 = 1$ and $i = n$ gives $u_n = k$.

Because of $\sum_{i=1}^{n} u_i = n(1+k)/2$ and $\sum_{i=1}^{n} u_i^2 = nk + (1-k)^2 n(2n-1)/6(n-1)$ formula (5) can be expressed as:

$$\mu = \frac{(1-k)^2(2n-1)+6k(n-1)}{3(1+k)(n-1)} \qquad (7)$$

Of special interest, of course, will be the case of a maximal divergence between the u_i's, that means: $k = 0$. From (7) we obtain:

$$\mu = \frac{2n-1}{3n-3} \quad (\text{for } k=0) \qquad (8)$$

This value often will be used for comparison purposes. In *Table 1* some numerical results for μ (by (7)) are summarized for different clone numbers n and different k's. One obtains the following main results:

Table 1. — Total yield μ for different clone numbers n and different k-values.

n \ k	0	0.10	0.30	0.50	0.70	0.90
2	1.000	0.918	0.838	0.833	0.876	0.953
3	0.833	0.796	0.775	0.806	0.867	0.952
4	0.778	0.755	0.755	0.796	0.865	0.952
5	0.750	0.734	0.744	0.792	0.863	0.951
6	0.733	0.722	0.738	0.789	0.862	0.951
7	0.722	0.714	0.734	0.787	0.862	0.951
8	0.714	0.708	0.731	0.786	0.861	0.951
9	0.708	0.703	0.728	0.785	0.861	0.951
10	0.704	0.700	0.727	0.784	0.861	0.951
15	0.691	0.690	0.722	0.782	0.860	0.951
20	0.684	0.686	0.719	0.781	0.860	0.951
25	0.681	0.683	0.718	0.780	0.859	0.951
30	0.678	0.681	0.717	0.780	0.859	0.951
35	0.677	0.680	0.716	0.779	0.859	0.951
40	0.675	0.679	0.716	0.779	0.859	0.951
45	0.674	0.678	0.716	0.779	0.859	0.951
50	0.674	0.678	0.715	0.779	0.859	0.951
75	0.671	0.676	0.714	0.779	0.859	0.951
100	0.670	0.675	0.714	0.778	0.859	0.951
125	0.669	0.675	0.714	0.778	0.859	0.951
150	0.669	0.674	0.713	0.778	0.859	0.951
175	0.669	0.674	0.713	0.778	0.859	0.951
200	0.668	0.674	0.713	0.778	0.859	0.951
300	0.668	0.674	0.713	0.778	0.859	0.951
400	0.668	0.673	0.713	0.778	0.859	0.951
500	0.667	0.673	0.713	0.778	0.859	0.951

Table 2. — Clone numbers n with 'difference (μ - $\lim_{n\to\infty} \mu$) less than g_1% of this limit' (for different k-values).

g_1 % \ k	0.00	0.10	0.30	0.50	0.70
20	4	3	2	2	2
10	6	5	3	2	2
5	11	9	5	3	2
2	26	20	10	5	3
1	51	38	19	9	4
0.5	101	74	37	16	6

1. For each given k (that means, for a given variability of the different u_i's, $i = 1,2,..,n$) we investigate $\mu = \mu(n)$: μ decreases with increasing clone number n.
   This decrease turns out to be most rapid for low numbers n, while for larger n only insignificant small changes of μ appear. Or mathematically formulated:
   $\lim_{n\to\infty} \mu(n)$ exists (for each k).
   μ from (7) can be expressed as:

$$\mu = \frac{2k}{1+k} + \frac{2-\frac{4}{n}}{1-\frac{1}{n}} \cdot \frac{(1-k)^2}{3(1+k)} \qquad (9)$$

From (9) we get directly the limit of μ:

$$\lim_{n\to\infty} \mu = \frac{2(1+k+k^2)}{3(1+k)} \qquad (10)$$

The limits increase with increasing k's.

Now we may ask for the clone number n for which μ has reached the limit so far that the difference will be lower than g_1% of this limit. These numbers n can be considered as necessary numbers of clones in clonal mixtures under the simplifying assumptions from above. For n we get the explicit expression:

$$n = 1 + \frac{(1-k)^2 \cdot 50}{(1+k+k^2) \cdot g_1} \qquad (11)$$

Some numerical results are presented in *Table 2.*

If we require an approach to the limit of at least 1% we have $n \cong 50$ (for a maximal variability of the u_i's, that means: $k = 0$). For an approach of at least 5% only $n \cong 10$ clones would be necessary.

This approach towards the limit seems to be an aspect of yield-level rather than an aspect of yield-stability. But, of course, insignificant changes of μ with increasing n would be also an aspect of yield-stability.

2. For each given n we now investigate $\mu = \mu(k)$. This aspect will be of special importance for the problem of phenotypic stability: μ-differences for different k-values (for a given number n) will characterize the phenotypic stability of the clonal mixture, because different k's represent the different variability between the clones involved. The lower these differences of μ for very dif-

Table 3. — Clone numbers n and k-values with $\mu = \mu(k) =$ minimum.

n	k	n	k	n	k
2	0.414	8	0.095	50	0.015
3	0.265	9	0.085	100	0.008
4	0.195	10	0.076	200	0.004
5	0.155	20	0.038	300	0.003
6	0.128	30	0.025	400	0.002
7	0.109	40	0.019	500	0.001

ferent k's, the higher will be the phenotypic stability of the clonal mixture.

From (7) and *Table 1* we may conclude: For each n the functions $\mu = \mu(k)$ show minima, which are located at very small numerical k-values. A necessary condition of an extreme point is $\frac{\partial \mu(k)}{\partial k} = 0$ which leads to

$$k = 1 - \sqrt{\frac{2n+2}{2n-1}} \qquad (12)$$

The second derivative turns out to be positive at this extreme point (12) and, therefore, it must be a minimum. We denote this k-value by k_{min}. Some numerical values are presented in *Table 3*.

These minima are located at very small numerical values of k — with the exception of very low clone numbers n. For $n \geq 8$ we have $k < 0.10$. Thus we may conclude: μ increases with increasing k (for clone numbers n which are not too low). That means: The total yields of clonal mixtures are the higher the more uniform the u_i's of the included clones.

If we consider the range of the possible μ-values (for any given n) as an indicator of stability we obtain (because of μ (for $k = 0$) $\leqq \mu$ (for $k = 1$)):

$$\text{range} = \mu \text{ (for } k = 1) - \mu \text{ (for } k = k_{min}) = 1 - \frac{(1-k_{min})^2(2n-1)+6k_{min}(n-1)}{3(1+k_{min})(n-1)} \qquad (13)$$

Application of (12) leads to

$$k_{min} = 1 - \sqrt{\frac{2n+2}{2n-1}} \qquad (14)$$

Combining (14) and (13) gives the range dependent on n. This range tends to the limit $\frac{1}{3}$ for $n \to \infty$. Again, we may ask for the clone number n for which this range has reached the limit so far that the difference will be lower than g_2% of this limit. Using this procedure these numbers n can be considered as necessary numbers of clones in clonal mixtures. We get the condition: 300 range = 100-g_2 which can be numerically solved for n (for any given percentage g_2). Some numerical results are:

g_2%	25	20	15	10	8	5	3	2	1
n	4	5	7	10	13	20	34	50	100

If we require an approach to the limit of at least 1% we have $n \cong 100$. This number reduces to $n \cong 20$ for $g_2 = 5$% and even to $n \cong 10$ for $g_2 = 10$%.

For any given clone number n and numerical values k_1 and k_2 with $k_1 \neq k_2$ we may investigate the yield-difference $\triangle$:

$$\triangle = \mu(n,k_1) - \mu(n,k_2) \qquad (15)$$

Using (7) we get by simple algebraic manipulations:

$$\triangle = (k_1-k_2) \cdot \left[\frac{2n-1}{3n-3} - \frac{2(n+1)}{3(1+k_1)(1+k_2)(n-1)} \right] \qquad (16)$$

Of special interest are the situations with $\triangle = 0$: Here different variabilities of the n clones (that means: different k's) will result in identical yields μ and that's, of course, an aspect of yield-stability. For example, if we use $k_1 = k$ and $k_2 = 0$ we may conclude: For an extension of the range of the u_i's of the n clones from 1-k to the maximal range 1 the yield μ remains unchanged. This increase of the differences between the clones doesn't result in corresponding yield-differences. From $\triangle = 0$ together with (16) we get the condition:

$$n = \frac{1}{2}\left(1 + \frac{3}{k_1+k_2+k_1k_2}\right) \qquad (17)$$

Table 4. — Clone numbers n for different values k_1 and k_2 leading to equal yields.

k_1 \ k_2	0	0.2	0.4	0.6	0.8	1.0
0.10	16	6	4	3	3	2
0.30	6	4	3	2	2	2
0.50	4	3	2	2	2	2
0.70	3	2	2	2	2	2
0.90	3	2	2	2	2	2

Some numerical results of (17) are presented in *Table 4*. For the especially interesting situation $k_1 = k$ and $k_2 = 0$ expression (17) can be simplified to:

$$n = \frac{3+k}{2k} \qquad (18)$$

(see, *Table 4*).

3. μ-differences for different clone numbers decrease with increasing k's. For example:

$$\left.\begin{array}{l} k = 0.10 : 0.673 \leqq \mu \leqq 0.918 \quad \begin{matrix}(\text{for } n = 500) & (\text{for } n = 2)\end{matrix} \\ k = 0.90 : 0.951 \leqq \mu \leqq 0.953 \quad \begin{matrix}(\text{for } n = 500) & (\text{for } n = 2)\end{matrix} \end{array}\right\} \qquad (19)$$

This aspect can be simply described by using the range v of the μ-values (for any given k). From (7) and (10) we obtain:

$$v = \mu \text{ (for } n=2) - \lim_{n \to \infty} \mu = \frac{1+k^2}{1+k} - \frac{2(1+k+k^2)}{3(1+k)}$$

which leads to

$$v = \frac{(1-k)^2}{3(1+k)} \qquad (20)$$

For some numerical values of k the ranges v are:

k	0.10	0.30	0.50	0.70	0.90
v	0.245	0.126	0.056	0.018	0.002

The range v decreases rapidly with increasing k's, that means with an increasing uniformity of the u_i's of the included clones. But, a decreasing range describes an increasing similarity of the possible yields, i.e. increasing stability.

The general expression can be simply derived: For any given k and clone numbers n_1 and n_2 with $n_1 \neq n_2$ we may study the yield-difference R:

$$R = \mu(n_1,k) - \mu(n_2,k) \qquad (21)$$

Using (7) we obtain:

$$R = \frac{(1-k)^2}{3(1+k)} \cdot \frac{n_2-n_1}{(n_1-1)(n_2-1)} = v \cdot \frac{n_2-n_1}{(n_1-1)(n_2-1)} \qquad (22)$$

We define $\frac{R}{v} = \lambda$ with λ = yield difference (expressed in units of the maximal difference for the corresponding k):

$$\frac{n_2-n_1}{(n_1-1)(n_2-1)} = \lambda \qquad (23)$$

If we proceed from (23) several possibilities leading to estimates of necessary clone numbers can be applied. For example, one approach may be: The yield-difference for different clone numbers n_1 and n_2 (expressed in v-units)

Table 5. — Necessary clone numbers n_1 (calculated by (24)) for different numerical values of t and λ (for explanations: see text).

t \ λ	0.01	0.03	0.05	0.10	0.15	0.20	0.25	0.30
1.2	19	8	5	4	3	3	3	2
1.4	31	12	8	5	4	3	3	3
1.6	40	15	10	6	4	4	3	3
1.8	46	17	11	6	5	4	4	3
2.0	52	19	12	7	5	4	4	4
2.2	56	20	13	7	6	5	4	4
2.4	60	21	14	8	6	5	4	4
2.6	63	22	14	8	6	5	4	4
2.8	66	23	15	8	6	5	4	4
3.0	68	24	15	8	6	5	4	4

shall be sufficiently small — for example $\lambda = 0.10$ — to guarantee stability. If we, additionally, want to double the clone number without changing the stability, (23) together with $\lambda = 0.10$ and $n_2 = 2n_1$ gives: $n_1 = 7$. If we require $\lambda = 0.05$ the result will be $n_1 = 12$.

In general, for $\frac{n_2}{n_1} = t > 1$ and (23) we obtain the result:

$$n_1 = \frac{\lambda(t+1)+(t-1)}{2\lambda t} \pm \sqrt{\left(\frac{\lambda(t+1)+(t-1)}{2\lambda t}\right)^2 - \frac{1}{t}} \qquad (24)$$

Some numerical results for n_1 are presented in *Table 5.* These numbers, therefore, can be characterized by the condition: realization of a certain, given yield-difference (measured by $\lambda = R/v$), which results from a certain, required increase of the number of clones (measured by $t = n_2/n_1$). These numbers n_1 can be considered as necessary numbers of clones in clonal mixtures, if definite numerical values for λ and t are available and well established. But, just this will be difficult — and this difficulty will be especially true of finding an appropriate numerical value for t. To choose appropriate values for λ will be easier, because this can be done by the acknowledgement of certain yield-differences (for different clone numbers) as being still in accordance with the required stability.

For example, if we use: $1.2 \leqq t \leqq 2$ and $0.03 \leqq \lambda \leqq 0.10$ we obtain $4 \leqq n_1 \leqq 19$ (see: *Table 5).*. Thus, for these parameter-intervals we may conclude a necessary number of approximately 20 clones. But this number must be considered as an upper bound for the necessary numbers of clones in clonal mixtures. For further numerical results we refer to *Table 5.*

Of special interest will be the case $n_2 = n_1 + 1$. From (23) it follows:

$$n_1 = 0.5 \pm \sqrt{\frac{1}{\lambda} + 0.25} \qquad (25)$$

Some numerical values of n_1 (by (25)) are presented in *Table 7.* The numerical value of λ gives the decrease in yield

Table 6. — Upper bounds (in %) (by (31)) for the yield-difference between the two cases of equally and unequally spaced u_i's, if this difference is expressed as a percentage of the yield for equally spaced u_i's.

n \ k	0.1	0.3	0.5	0.7	0.9
5	7.52	3.80	1.58	0.46	0.04
10	2.08	1.02	0.42	0.12	0.01
15	0.94	0.46	0.19	0.05	0.00
20	0.54	0.26	0.11	0.03	0.00
30	0.24	0.12	0.05	0.01	0.00
50	0.09	0.04	0.02	0.00	0.00
100	0.02	0.01	0.00	0.00	0.00

Table 7. — Necessary numbers n_1 of clones with λ = yield-decrease (expressed in units of the maximal yield-difference) caused by one additional clone.

λ	n_1	λ	n_1	λ	n_1	λ	n_1
0.0001	101	0.001	33	0.03	7	0.15	4
0.0005	46	0.005	15	0.05	5	0.20	3
0.0008	36	0.01	11	0.10	4	0.25	3

(expressed in v-units), if the number of clones will be increased by one clone. This yield effect caused by only one additional clone must be very low. Therefore, only very small values of λ are of any practical interest. For numerical results we refer to *Table 7.*

Discussion

A critical discussion of the numerical results on necessary clone numbers primarily should be deal with an investigation of the numerous simplifying assumptions and their possible importance and resulting restrictions. In the preceding theoretical investigations two parameters have been introduced to provide a quantitative analysis: A 'survival-parameter' describing shifts of the composition of the clonal mixture and a 'yielding ability-parameter' characterizing the different yielding potential of the clones (independent on the selective effects). This general 'two-parameter-approach' (survival and yielding ability) has been simplified in this paper by assuming a proportionality between these two characters. With regard to long-living organisms like forest trees this assumption seems to be justified. Many natural selection processes and the different non-systematic artificial thinning procedures too are in accordance with this simplyfying assumption.

The assumption "equal proportions of the clones in the initial composition of the clonal mixture" seems to be no serious restriction:

1. With regard to aspects of designing and silvicultural practices equal proportions will be used almost completely. A realization of definite unequal proportions would result in considerable practical difficulties.
2. Although mixing effects in mixtures may be dependent on the proportions of the clones in the mixture a systematic utilization of this dependence in practical breeding programs will be nearly impossible. Such a purpose would require continuous extensive experimental investigations to find out the optimal proportions. In practical breeding this expensive work cannot be performed. This aspect will be further strengthened, if we compare the gain which can be obtained by using the optimal proportions with the necessary expense for it.
3. If we denote the optimal proportion of the clone i in the clonal mixture with $(f_i)_{opt}$ we have:

$$\sum_{i=1}^{n} \left[\frac{1}{n} - (f_i)_{opt}\right] = 0 \qquad (26)$$

Therefore, some of the optimal proportions are above and some are below the value $\frac{1}{n}$ in the case of equal proportions of the clones in the mixture. If we calculate the yield improvements caused by deviations of the composition of the clonal mixture from the optimal proportions we may conclude: Because of the different sign of the terms $[\frac{1}{n} - (f_i)_{opt}]$ some of the effects will be cancelled out.

With increasing clone number n the frequencies $\frac{1}{n}$ de-

crease. The same will be true of the $(f_i)_{opt}$, if we suppose that not only a few clones dominate in the clonal mixture. But just this should be avoided to maintain the genetic diversity on a sufficient level. But, if $(f_i)_{opt}$ as well as $\frac{1}{n}$ decrease with increasing clone numbers n in the consideration of the differences $[\frac{1}{n} - (f_i)_{opt}]$ random effects will be of an increasing importance. The compensating effects which have been mentioned before can be further strengthened by these considerations.

Summarizing these different arguments we may conclude that the problem of optimal proportions of the clones in the clonal mixture will be of reduced importance. Therefore, the assumption $f_i = \frac{1}{n}$ for each i seems to be obvious.

Another simplification shall be mentioned: The 'total yield' has been expressed throughout by (5) in NL_{max}-units. This implies the assumption of constant values for $N \cdot L_{max}$ in spite of varying u_i-parameters. If we proceed from a constant final number of plants the assumption NL_{max} = const implies an equal yielding-ability of the clones with the highest frequency in the final population.

But, this simplification will be no serious restriction for practical applications, if we are only interested in an approximate and rough numerical level of necessary clone numbers. Furthermore, in spite of varying u_i-parameters very often the same clone will be the dominating clone at the final stage of production. Preceeding from a constant final number of plants in these situations the assumption NL_{max} = const will be always comes true.

The extremely simplified deterministic approach of equally spaced u_i-values in the interval from k to 1 will not alter the rough numerical magnitude of the necessary clone numbers for clonal mixtures too much — compared to the results under the more realistic assumption of unequally distributed u_i's:

$$u_i^* = \underbrace{1 - \frac{1-k}{n-1}(i-1)}_{= u_i \text{ equally spaced value}} + \underbrace{E_i}_{\text{deviation}} \qquad (27)$$

The lowest and largest value of u_i^* must be k and 1 respectively. Thus we have: $E_1 = E_n = 0$. From (5) together with (27) one obtains for the total yield μ^*:

$$\mu^* = \frac{\sum_{i=1}^{n} (u_i^*)^2}{\sum_{i=1}^{n} u_i^*} = \frac{nk + \frac{(1-k)^2 n(2n-1)}{6(n-1)} + 2\sum_{i=1}^{n} E_i u_i + \sum_{i=1}^{n} E_i^2}{\frac{n(1+k)}{2} + \sum_{i=1}^{n} E_i} \qquad (28)$$

We may assume an independence between E_i and u_i, which leads to $2\sum_{i=1}^{n} E_i u_i = (1+k) \cdot \sum_{i=1}^{n} E_i$. Furthermore, the E_i's will be positive or negative. To get a first, rough approximation we may assume: $\sum_{i=1}^{n} E_i = 0$. From (28) it follows:

$$\mu^* = \mu + \frac{\sum_{i=1}^{n} E_i^2}{n(1+k)} \qquad (29)$$

The difference $\mu^*-\mu$ can be expressed as a percentage of μ and will be denoted by diff (%): (7) together with (29) gives:

$$\text{diff}(\%) = 100 \cdot \frac{\mu^*-\mu}{\mu} = \frac{100}{n} \cdot \frac{\sum_{i=1}^{n} E_i^2}{k + \frac{(1-k)^2(2n-1)}{6(n-1)}} \qquad (30)$$

By (6) we have:

$$u_{i+1} - u_i = \frac{1-k}{n-1} \qquad (31)$$

Together with $E_1 = E_n = 0$ from (30) we obtain a very rough upper bound of diff(%) by assuming $\sum_{i=1}^{n} E_i^2 \leq (n-2)\left(\frac{1-k}{n-1}\right)^2$:

$$\text{diff}(\%) \leq \frac{100(n-2)}{n(n-1)^2} \cdot \frac{1}{\frac{2n-1}{6(n-1)} + \frac{k}{(1-k)^2}} \qquad (32)$$

Some numerical values of this upper bound are presented in Table 6. For almost all situations these upper bounds are extremely low. Only for very small clone numbers n and simultaneously very low k's these upper bounds are somewhat larger. But these situations are of little interest for practical applications. Thus, we may conclude that the difference $\mu^*-\mu$ will be sufficiently low. Therefore, the assumption of equally spaced u_i-values in the interval from k to 1 will be no serious restriction in this simple deterministic approach.

In this paper numerical values for the necessary number of clones in clonal mixtures have been calculated by using very different approaches, for example:

1. $|\mu(n)$-limit $|$ lower than g_1% of this limit.
2. | range of μ(k)-maximal range | lower than g_2% of this maximal range.
3. Calculation of clone numbers by assuming certain numerical values for t and λ.

According to these approaches a critical comment must be added: Certain numerical values for these quantities are arbitrary assumptions. What are the appropriate numerical values of g_1, g_2, t and λ? Only cogent arguments leading to definite numerical values for these parameters would dissipate these objections.

Literature

Burdon, R. D.: The roles and optimal place of vegetative propagation in tree breeding strategy. Proceedings of the IUFRO Joint Meeting of Working Parties on Genetics about Breeding Strategies including multiclonal varieties. Sensenstein Sept., 66—83 (1982). — Hall, R. B.: Breeding Trees for Intensive Culture. Proceedings of the IUFRO Joint Meeting of Working Parties on Genetics about Breeding Strategies including multiclonal varieties. Sensenstein Sept., 182—193 (1982). — Heybroek, H. M.: Possibilities of clonal plantations in the 1980s and beyond. K. A. Longman (ed.): Vegetative propagation of trees in the 1980s, 21—22. C.F.I. Occassional papers No. 15 Dept. of Forestry, University of Oxford (1981). — Heybroek, H. M. and van Tol, G.: Knowledge of sites and of site-requirements will reduce the optimal number of genotypes in a mixture. Proceedings of the IUFRO Joint Meeting of Working Parties on Genetics about Breeding Strategies including multiclonal varieties. Sensenstein Sept., 113—114 (1982). — Huehn, M.: Theoretische Untersuchungen zur Problematik der Mindestklonzahl in Mehrklonsorten. Teil I: Einführung in die Problematik, Literaturübersicht sowie Anmerkungen zur Zielsetzung der Untersuchungen. Hessisches Ministerium für Landesentwicklung, Umwelt, Landwirtschaft und Forsten Wiesbaden, 1—34 (1984). — Kampmeijer, P. and Zadoks, J. C.: EPIMUL, a simulator of foci and epidemics in mixtures of resistant and susceptible plants, mosaics and multilines. Simulation Monographs, Centre for Agricultural Publishing and Documentation Wageningen (1977). — Kleinschmit, J.: A program for large scale cutting propagation in Norway spruce. IUFRO-Meeting New Zealand, November (1973). — Kleinschmit, J.: Considerations regarding breeding programs with Norway spruce (*Picea abies* Karst.). Proceedings IUFRO Joint Meeting of Working Parties on Population and Ecological Genetics, Breeding Theory and Progeny Testing, Stockholm (Dep. For. Gen., Roy. Coll. of Forestry), 41—58 (1974). — Kleinschmit, J.: Limita-

tions for restriction of the genetic variation. Silvae Genetica **28**, 61—67 (1979). —— KLEINSCHMIT, J.: Concepts and Experiences in clonal plantations of Conifers. Proceedings of the 19th Meeting of the Canadian Tree Improvement Association. Part 2: Symposium on 'Clonal Forestry: its impact on tree improvement and our future forests' (Editors: ZSUFFA, RAUTER and YEATMAN). Toronto, Ontario 26—56 (1983). —— KRUSCHE, D.: Host-parasite interaction and the number of clones in a clonal mixture. Proceedings of the IUFRO Joint Meeting of Working Parties on Genetics about Breeding Strategies including multiclonal varieties. Sensenstein Sept., p. 171 (1982). —— LIBBY, W. J.: What is a safe number of clones per plantation? In: Resistance to diseases and pests in forest trees. Proceedings 3. Int. Workshop on the Genetics of Host-Parasite interactions in Forestry, Wageningen Sept., 342—360 (1980). —— LINDGREN, D.: Possible advantages and risks connected with vegetative propagation for reforestation. In: ELIASSON *et al.* (eds.): Vegetative propagation of forest trees — Physiology and practice. Institute of Forest Improvement and Department of Forest Genetics, Swedish College of Forestry, Uppsala, 9—16 (1977). —— MARSHALL, D. R. and BROWN, A. H. D.: Stability of performance of mixtures and multilines. Euphytica **22**, 405—412 (1973). —— MARSHALL, D. R. and ALLARD, R. W.: Performance and stability of mixtures of grain sorghum. I. Relationship between level of genetic diversity and performance. Theoretical and Applied Genetics (TAG) **44**, 145—152 (1974). —— NANSON, A.: Closing topics. Proceedings of the IUFRO Joint Meeting of Working Parties on Genetics about Breeding Strategies including multiclonal varieties, Sensenstein Sept., 228—231 (1982). —— OSTERGAARD, H.: Predicting Development of Epidemics on Cultivar Mixtures. Phytopathology **73**, 166—172 (1983). —— TIGERSTEDT, P. M. A.: The application of ecological genetics principles to forest tree breeding. Silvae Genetica **23**, 62—66 (1974). —— TRENBATH, B. R.: Interactions among diverse hosts and diverse parasites. Annals of the New York Academy of Sciences Vol. **287**, 124—150 (1977).

Clone Certification by use of Cortical Monoterpenes as Biochemical Markers

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Summary

Monoterpenes can be useful biochemical markers for certifying clones in seed orchards. Cortical oleoresin from buds in the top branches of 81 clones in a research seed orchard were sampled for monoterpene composition to use in distinguishing between clones. Of 3240 possible paired combinations of clones, 2623, 2202, 880, and 302 combinations of clones differed in the composition of one, two, three, and four of the five major monoterpenes respectively. Only 19%, or 617, of the possible combinations of clones could not be separated at all. Beta-phellandrene separated the greatest number of combinations of clones and limonene, the least. Scions from a commercial seed orchard had been mislabeled in establishing a second and third seed orchard and errors were verified by using monoterpene analysis. It is recommended that trees in research seed orchards be subjected to monoterpene analysis to verify correct labeling so that errors are not perpetuated in breeding and genetic studiees or in commercial seed orchard establishment.

Key words: α-pinene, β-pinene, β-phellandrene, seed orchard, ramets, oleoresin, gum.

Zusammenfassung

Monoterpene können nützliche biochemische Marker zur Zertifizierung von Klonen in Samenplantagen sein. In einer Versuchssamenplantage mit 81 Klonen wurde Rindenbalsam von Terminalknospen auf den Gehalt an Monoterpenen sowie deren Eignung zur Klonunterscheidung untersucht. Von 3240 möglichen paarweisen Klonkombinationen unterschieden sich die Kombinationen 2623, 2202, 880 und 302 in 1, 2, 3 bzw. 4 der 5 Haupt-Monoterpene. Nur 19% oder 617 der möglichen Kombinationen von Klonen konnten überhaupt nicht getrennt werden. Anhand von Beta-Phellandren konnte die größte, von Limonen die geringste Anzahl der Klonkombinationen getrennt werden. Durch falsche Kennzeichnung von Pfropfreisern in einer kommerziellen Samenplantage waren in den nachgezogenen Plantagen Fehler entstanden, die durch die Monoterpen-Analyse aufgedeckt wurden. Es wird empfohlen, daß Bäume in Versuchssamenplantagen einer Monoterpen-Analyse unterzogen werden, um die korrekte Kennzeichnung sicherzustellen, so daß Fehler sich nicht bis in die Züchtung und in genetische Studien oder in eingerichtete kommerzielle Samenplantagen hinein fortsetzen.

Introduction

Scions may be accidentally mislabeled in breeding operations or in grafted seed orchards. Unless there are strong morphological differences in the vegetative structures of scions or in the morphology of cones and seeds, the errors can go unnoticed. And even if an error becomes apparent, the mislabeled material must be identified or discarded. A rapid and nondestructive means of clone certification is needed to avoid this problem.

Monoterpene composition of cortical or foliar oleoresin holds much promise for identifying conifer clones in breeding programs and seed orchards. It has been successfully used to identify cultivars of *Picea pungens* ENGELM. (ROTTINK and HANOVER, 1972) and *Juniperus horizontalis* MOENCH (FRETZ, 1977). Monoterpenes have also been used to separate clones of *Pseudotsuga menziesii* (MIRB.) FRANCO (RADWAN and ELLIS, 1975), *Pinus sylvestris* L. (THORIN and NOMMIK, 1974), and *Picea abies* (L.) KARST. (ESTEBAN *et al.*, 1976). Research on cortical monoterpenes has shown that the nongenetic within-tree variation in the monoterpene composition is much less than the variation among trees of a species (HANOVER, 1966). Thus, by identifying monoterpene phenotypes of trees one may be able to certify ramets from clones. HANOVER (1966) found that intraclass correlation coefficients for monoterpene composition in *Pinus monticola* DOUGL. ex D. DON were high. He suggested

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