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# Determination of the Minimum Number of Stool Bed Ortets Required to Capture a Desirable Genotype from Full-Sibling Family Crosses

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**ABSTRACT:** *Two important questions for clonal forestry are: (1) how many ortets must be established to ensure that one or more of the best genotypes in a family will be available for field tests and plantation establishment; and (2) how certain can one be that at least one top genotype will be present in a sample of n ortets. In this study, we calculated the level of confidence (LOC) in having included one or more desirable, rootable genotypes in a random sample of n ortets from a full-sibling family. We also calculated the number of unique ortets required to achieve a given LOC in having included one or more desirable, rootable genotypes in a sample. In general, when the sample size is small, either because the original number of ortets was low or because of poor rootability, the LOC is lower. When rootability is low or when only a small percentage of the possible genotypes is considered desirable, the original number of ortets required to achieve a given LOC is higher. Both LOC and sample size are highly influenced by the target number of desirable genotypes to be captured in a sample of ortets. South. J. Appl. For. 27(3):160–163.*

**Key Words:** Sample size, ortet, stool bed, vegetative propagation.

Clonal forestry can have a significant advantage over seedling forestry if only the best clones (i.e., those whose genotypic values are well above their respective family means) are propagated for deployment. For many species, however, the current practice is to establish ortets using individuals whose genotypic values are unknown, but whose full-sibling families have been progeny-tested (Frampton et al. 1999). Clonal performance is expected to be distributed about the full-sibling family mean, but the performance of any individual clone cannot be predicted until field testing occurs. The probability of identifying top individuals increases as the number of ortets evaluated within a full-sibling family increases, but so does the cost of the testing program. Therefore, determining a sample size that is cost-efficient, yet gives an acceptable level of confidence in having included one of the best clones within a family, is critical to the success of the program. In the present study, we determined two values: (1) the level of confidence in having included one or more desirable genotypes in a random sample of seedling-derived

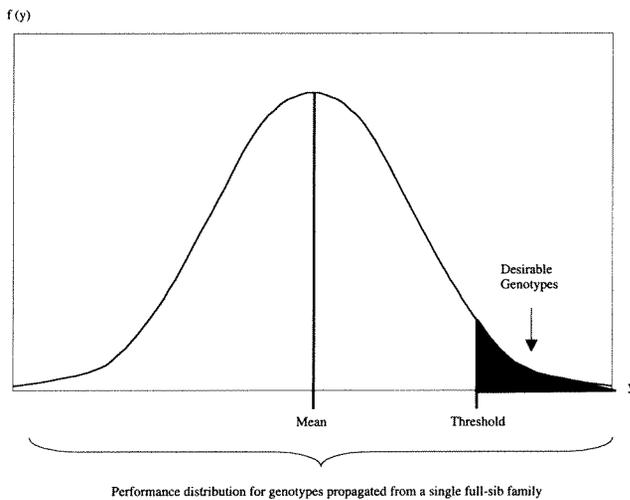
ortets from a full-sibling family; and (2) the sample size necessary to achieve a given level of confidence in having included one or more desirable genotypes in a family of ortets.

## Methods

### Probability of Including a Desirable Genotype

The number of individuals within a family is limited only by the ability to produce seeds and seedlings from a desired cross. Thus, there are potentially an infinite number of phenotypes within a family. If one assumes that the genotypic values of those phenotypes are normally distributed about the family mean, then the proportion of phenotypes considered desirable (i.e., the best) can be calculated from the proportion of genotypic values above some threshold (Figure 1). This threshold can be defined either as some best percentage of individuals or by a genotypic value some number of standard deviations above the family mean. If one also assumes that there is no correlation between an ortet's genotype for clonal performance and its rooting ability, then the probability of a rootable ortet having a desirable genotype (*dgr*) is equal to the joint probability of randomly sampling a desirable genotype from the family and of the genotype rooting.

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**Figure 1. The probability of a random ortet having a genotype above some threshold value is determined from the properties of a normal distribution and is equal to the area to the right of the threshold under the curve.**

$$\text{Probability (dgr)} = (\text{PDG} * \text{rootability}) \quad (1)$$

where *PDG* is the percentage of the family with desirable genotypes, and *rootability* is the proportion of ortets within the full-sibling family having acceptable rooting percentages. The probability of a rootable ortet not having a desirable genotype is then:

$$\text{Probability (no dgr)} = 1 - (\text{PDG} * \text{rootability}) \quad (2)$$

If each ortet established in the stool bed is viewed as an attempt to include a desirable, rootable genotype, the probabilities calculated in Equations (1) and (2) can be used to determine the binomial probability of including any number of desirable, rootable genotypes in a sample of *n* ortets:

$$\text{Probability (inclusion)} = \binom{n}{x} [\text{Prob. (dgr)}]^x [\text{Prob. (no dgr)}]^{n-x} \quad (3)$$

where *x* is the target number of desirable, rootable clones to be identified and *n* is the total number of unique ortets initially established in the stool bed. More importantly, Equations (1) and (2) can also be used to calculate the probability or risk of not including at least *x* *dgrs* in a random sample of ortets:

$$\text{Probability (noninclusion)} = \sum_{r=0}^{x-1} [\text{Prob. (dgr)}]^r [\text{Prob. (no dgr)}]^{n-r} \quad (4)$$

## Level of Confidence

If a sample of *n* different phenotypes is taken at random from a family, the level of confidence (LOC) in having included at least *x* desirable genotypes in a sample is equal to 1 minus the probability of noninclusion or:

$$\text{LOC} = 1 - \sum_{r=0}^{x-1} \binom{n}{r} [\text{Prob. (dgr)}]^r [\text{Prob. (no dgr)}]^{n-r} \quad (5)$$

When *x* = 1, Equation (5) reduces to:

$$\text{LOC} = 1 - [\text{Probability (no dgr)}]^n \quad (6)$$

## Sample Size

The number of unique phenotypes (ortets within a family) required to achieve a given LOC in having included at least one desirable ortet can be estimated by solving Equation (6) for *n*:

$$n = \frac{\ln[1 - \text{LOC}]}{\ln[1 - \text{PDG} * \text{Rootability}]} \quad (7)$$

Calculating the sample size required to achieve a LOC in having included at least *x* > 1 genotype is not as simple since Equation (5) cannot be solved for *n* directly. Instead, the number of unique phenotypes required to achieve a given LOC for *x* > 1 must be determined by entering increasing levels of *n* into Equation (5) until the desired LOC is achieved.

## Results and Discussion

When compared on average, clonal performance is similar to that of seedlings from the same full-sibling family (Frampton and Foster 1993, Stelzer et al. 1998). However, the performance of individual clones can exceed that of full- and half-sibling selections. For example, Mullin et al. (1992) estimated that 22.6% gains in 5 yr height growth could be achieved with the deployment of the top 1% of black spruce [*Picea mariana* (Mill.) B.S.P.] clones while gains of only 13.1% and 16.7% were attainable with the deployment of the top 1% of full- and half-sibling family selections, respectively.

Wricke and Weber (1986) demonstrated that the risk of not finding phenotypically favorable individuals could be minimized by optimizing the number of crosses and the number of progeny per cross. Weber (1979) also showed that these numbers could be optimized for a given population size, i.e., that of a breeding program. Both of these studies assumed there was a probability that the crosses evaluated were not favorable, and thus concluded that the number of crosses should be maximized and the number of progeny per cross should be minimized. In our study, we assumed that the breeder has tested the crosses and wants to find the best possible genotypes within superior full-sibling families.

We based our calculations not only on the probability of an ortet having a desirable genotype but also on the probability that the desirable genotype would root. Because the rooting ability of different clones has been shown to vary as much if not more within families as between them (e.g., Foster 1990, Fries and Kaya 1997), we assumed that some desirable genotypes fail to root at acceptable levels and are discarded. Therefore, establishing an ortet with a desirable genotype would not guarantee that the genotype would be available for clonal testing. We assumed that there was no correlation between an ortet's genotype and its ability to root because there have been no long-term data reported on the subject. This allowed us to assume that the performance distribution for rootable clones would be normally

**Table 1a. Levels of confidence (%) in having captured at least one ortet with a rootable genotype in the top 1, 5, and 10% of clones based on different sample sizes and family rootabilities.**

Rootability (%)	n = 100			n = 50			n = 10		
	PDG: 10%	5%	1%	10%	5%	1%	10%	5%	1%
100	~100	99	63	99	92	39	65	40	10
90	~100	99	60	99	90	36	61	37	9
80	~100	98	55	98	87	33	57	34	8
70	~100	97	50	97	83	30	52	30	7
60	~100	95	45	95	78	26	46	26	6
50	99	92	39	92	72	22	40	22	5
40	98	87	33	87	64	18	34	18	4
30	95	78	26	78	53	14	26	14	3
20	87	63	18	64	39	10	18	10	2
10	63	39	10	39	22	5	10	5	1

**Table 1b. Levels of confidence (%) in having captured at least two ortets with a rootable genotype in the top 1, 5, and 10% of clones based on different sample sizes and family rootabilities.**

Rootability (%)	n = 100			n = 50			n = 10		
	PDG: 10%	5%	1%	10%	5%	1%	10%	5%	1%
100	~100	96	26	97	72	9	26	9	0
90	~100	94	23	95	66	7	23	7	0
80	~100	91	19	92	60	6	19	6	0
70	99	87	16	87	53	5	15	5	0
60	98	81	12	81	44	4	12	3	0
50	96	72	9	72	36	3	9	2	0
40	91	60	6	60	26	2	6	2	0
30	81	44	4	44	17	1	3	1	0
20	60	26	2	26	9	0	2	0	0
10	26	9	0	9	3	0	0	0	0

distributed (Figure 1). Fries and Kaya (1997) found a positive genetic correlation between rooting percentage and height growth during the rooting period but they did not report whether this correlation held once the rooted cuttings were transferred to the field. A positive correlation would skew the distribution in Figure 1 to the right, and lower the sample size required to achieve a given LOC. A negative correlation would skew the distribution to the left and increase the necessary sample size.

With the joint probability determined, it was possible to calculate the LOC in having included a variable number of desirable genotypes in a random sample of n ortets and the sample size necessary to achieve a given LOC. The probability of not including *at least x* desirable, rootable genotypes was used to estimate risk rather than the probability of not including *exactly x* *dgrs* because it took into account the probability that samples could contain more than *x dgrs*. Examples of the levels of confidence for *x = 1* and *x = 2* when *n = 10, 50, and 100* are given in Table 1. Note that varying the target number of desirable genotypes to be captured in a sample had a dramatic impact on

the LOC. Varying the PDG and the rootability also had a dramatic impact on the LOC, especially when either value was low. Table 2 contains the sample size necessary to achieve a given LOC for *x = 1* and *2*. For *x = 2*, increasing levels of *n* were entered into an iterative algorithm for Equation (5) until the designated LOCs were achieved (Table 2b).

### Practical Examples

The analyses derived in this study have many applications in clonal forestry. For example, the decision of whether or not to include a particular family in clonal tests could be based on the likelihood that a desirable genotype is available for testing. Equation (5) can be used to determine the LOC in having captured one or more of the best genotypes in a family given the number of rootable ortets available (Figure 2). A high LOC indicates that the number of ortets is sufficient to begin testing. However, a low LOC suggests that the risk of not capturing one of the best genotypes is high and more rootable ortets should be

**Table 2a. Initial number of ortets within a full-sibling family required to capture at least one rootable genotype in the top 1%, 5%, and 10% with an LOC of 95% to 99% when rootability is 10% to 100%.**

Rootability (%)	LOC = 95%			LOC = 99%		
	PDG: 10%	5%	1%	10%	5%	1%
100	29	59	299	44	90	459
90	32	66	332	49	101	510
80	36	74	373	56	113	574
70	42	85	427	64	130	656
60	49	99	498	75	152	766
50	59	119	598	90	182	919
40	74	149	748	113	228	1,149
30	99	199	998	152	305	1,533
20	149	299	1,497	228	459	2,301
10	299	598	2,995	459	919	4,603

**Table 2b. Initial number of ortets within a full-sibling family required to capture at least two rootable genotypes in the top 1%, 5%, and 10% with an LOC of 95% to 99% when rootability is 10% to 100%.**

Rootability (%)	LOC = 95%			LOC = 99%		
	PDG: 10%	5%	1%	10%	5%	1%
100	46	93	473	64	130	662
90	51	104	526	71	145	735
80	58	117	592	81	164	827
70	66	134	676	92	187	946
60	78	157	789	108	219	1,104
50	93	188	947	130	263	1,325
40	117	236	1,185	164	330	1,657
30	157	315	1,580	219	440	2,210
20	236	473	2,371	330	662	3,317
10	473	947	4,742	662	1,325	6,636

obtained either by increasing the rootability of the family or by establishing a greater number of ortets.

LOC analyses can also be used in decisions regarding the allocation of stool bed space. If a breeder has the resources to establish a total of  $n$  ortets and those  $n$  ortets are to be divided among multiple families, Equation (7) can be used to estimate the different sample sizes required by each family (depending on their rootabilities and PDGs) to achieve a given LOC. Sample sizes could then be tailored to individual families based on rootability and the percentage of family genotypes considered desirable. Note that the PDG could be variable depending on the objectives of the breeding program. If a threshold value were to be set at a given genotype value rather than a set percentage of genotypes (e.g., the top 10% from each family), the percentage of individuals with desirable genotypes in each family would depend on the family means and within-family variances. Families with lower means or lower variances may have fewer individuals whose genotypic values exceed the desired threshold. These families would then require the establishment of a greater number of ortets to achieve the same LOC as a family with a higher mean or greater within-family variance.

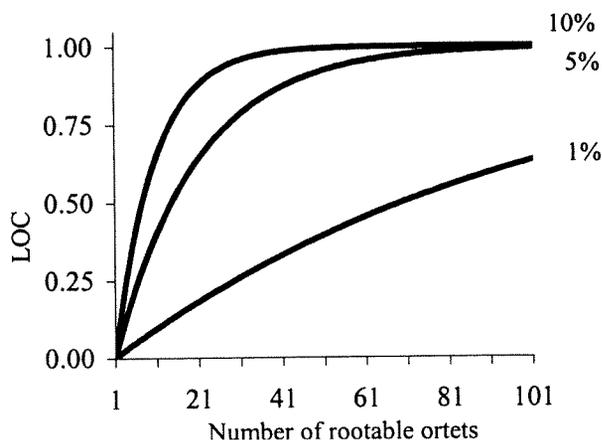
## Summary

This study provides information that can be used by plant propagators to determine appropriate numbers of stool bed

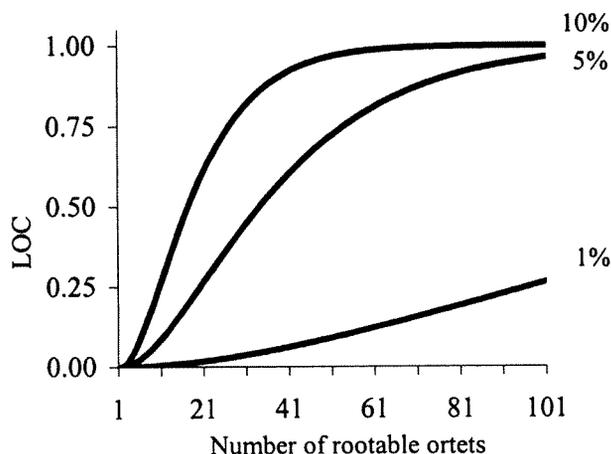
ortets to establish from individual, untested genotypes to ensure that desirable genotypes are available for testing and plantation establishment. Our results can also be used to allocate efficiently stool bed space by estimating appropriate sample sizes for different full-sibling families. The same rationale applies to any system of vegetative propagation where clones are produced prior to field testing.

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**Figure 2a. The level of confidence (LOC) in having captured one or more desirable genotypes in the top 1, 5, and 10% of clones given varying numbers of rootable ortets.**



**Figure 2b. The level of confidence (LOC) in having captured two or more desirable genotypes in the top 1, 5, and 10% of clones given varying numbers of rootable ortets.**