# PREDICTING LOBLOLLY PINE SEEDLING PERFORMANCE FROM SEED PROPERTIES

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Abstract.- Seed properties affect seedling performance, and may confound evaluations of progeny tests and determinations of the best seed handling practices. The total seed weight, coat weight, gam/emb weight, lipid content, calories per gam/emb, days to seedling establishment, and the weights of the seedlings were determined for the seeds of 19 clones of loblolly pine. There were large variations among clones in all seed and seedling properties measured. Various combinations of seed properties accounted for as much as three fourths of the variation in seedling weight.

## INTRODUCTION

Performance of the same progenies in different field tests is often not consistent. One source of variation could arise from differences in seed properties and behavior among clones. This paper reports on clonal variations in seed properties of loblolly pine *(Pinus taeda* L.) and their effects on the initial performance of progenies.

In an investigation of loblolly pine seeds, Perry and Hafley (1981) examined seed weight, embryo condition, seed coat thickness, stratification requirements, and the number of days required to shed the seed coat. At best, these factors only accounted for about 20% of the variability in seedling size. To explain more of this variation, it is proposed here that differences in loblolly pine seedlings are associated with differences in energy content of the gametophyte and embryo of the seeds, as well as other seed properties.

The objectives of this study were to:

1. Identify genetic differences in the following seed properties of loblolly pine: seed coat, gametophyte and embryo, and total seed weight; total lipid content; energy content (calories per seed); and number of days to seedling establishment,

2. Measure the performance of the seedlings produced from the seeds, and

3. Correlate seedling performance with seed properties.

## MATERIALS AND METHODS

Seeds were obtained from open pollinated clones of loblolly pine in the Weyerhaeuser Corporation orchard in Washington, North Carolina. Samples from the seedlots of 10 clones from the 1981 collection and 9 clones from the 1982 collection were obtained.

Fifty seeds were chosen randomly from each seedlot. The seed coat was removed. Each seed coat and each combined gametophyte and embryo (gam/emb) were weighed and saved for subsequent measurements.

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The total lipid of each seed was extracted by a modification of the procedure outlined by Christie (1982). Each gam/emb was crushed in 2 ml of chloroform:methanol (2:1) and treated with a sonic disintegrator for about 30 seconds to insure complete homogenization and dissolution of all lipid. The resulting mixtures were filtered into weighed tubes. Distilled water, equal to one-quarter of the total volume of the filtrate, was added to each filtrate (approximately 0.38 ml water to 1.52 ml filtrate). The solvents partitioned into an aqueous upper phase and an organic lower phase. The lower phases, containing the extracted lipid, were dried in a vacuum dessicator connected to a dry-ice/acetone cold trap and a vacuum pump. The amount of lipid in each dried extract was determined gravimetrically. A correction factor which accounted for the amount of lipid lost from a gam/emb during the extraction procedure was determined by performing the entire extraction process on 25 seed lipid aliquots of known weight, as if each were a single seed.

Seven clones representing the entire range of lipid concentration (gm lipid per gm gam/emb) were chosen for measurement of total energy content using a bomb calorimeter. One-hundred seeds of each clone were decoated and divided into two groups of 50 gam/embs each and weighed. Each group was weighed, placed in a gelatin capsule, and burned. The mean calories per gram of gam/emb determined for each of these clones were regressed against the lipid concentrations. This regression equation was then used to estimate the calories per gram of gam/emb of the seeds of all clones.

Forty-nine stratified seeds and 49 unstratified seeds of each clone were planted at the same time in "Cone-tainers" (1 seed in each). Seeds were stratified by placing them in a pipette washer with cold running water for 48 hours and then storing them for 40 days at 7 C. They were arranged in random groups of 7 in racks in a greenhouse (only stratified or unstratified seeds in each group), watered when necessary, and fertilized approximately every 10 days. The number of days to seedling establishment (DTE) was recorded for each seed and the mean DTE was calculated for each seedlot. Seedling establishment is defined here as the number of days from planting to the day of hypocotyl straightening.

An establishment value (EV) was calculated for each seedlot by substituting seedling establishment for germination into the formula for the germination value proposed by Czabator (1962). The establishment value is a measure of both the speed and the completeness of seedling establishment. Calculations were made at 30 days for unstratified seedlots and 20 days for stratified seedlots.

After 75 days from planting, the seedlings from stratified seeds were harvested (both roots and shoots) because their roots had almost filled the containers and there was no further seedling establishment. The seedlings from unstratified seeds were harvested 97 days after planting because there was still some seedling establishment after 75 days. All seedlings were oven-dried at 80 C for 24 hours and then weighed.

## RESULTS

### Seed and Seedling Properties

There were significant differences among clones in all seed properties measured (Table 1).

The loss of lipid in the extraction procedure was determined to be 10% of the actual lipid weight and the weight of lipid from each gam/emb was corrected accordingly. The gam/embs varied in lipid concentration from 36% to 70% (mean = 47%).

The regression used to estimate the calories per gram of gam/emb from lipid concentration had an R of 0.88.

Significant differences were evident among mean DTEs of both stratified and unstratified seeds of many clones (Table 2). Stratification reduced the mean DTE and its variance by 59% and 70%, respectively. As a result, there were fewer differences among clones in DTE for stratified seeds.

### Prediction Equations

Unstratified seeds.- Every combination of mean DTE and means of seed properties was examined by regression analysis for prediction of seedling weight (Table 3). DTE alone accounted for 63% of the variation in seedling size. When the calories per gam/emb were added to the relationship, 76% of the variation could be explained.

The number of calories per gam/emb was closely related to the gam/emb weight (R = 0.99), probably because the calculation of total calories per individual gam/emb was very sensitive to even small changes in the gam/emb weight. When the lipid concentration was halved, only a 20% increase in the gam/emb weight was necessary to maintain the same total number of calories. Because of this large dependence, the gam/emb weight could be substituted for the calories per gam/emb$_2$ in the regression without affecting the ability to predict seedling size (R = 0.76). These were the only models which accounted for a large amount of the variation in the size of seedlings from unstratified seeds.

Model 2 supports the hypothesis that seedling weight is affected by the energy content of the seed. However, Model 3 is more useful for predictive purposes. It is easier to weigh the gam/embs than to burn them.

Combinations of variables for regression models were reexamined, substituting EV for DTE. There were no improvements in the regression coefficients for the previous models and no new models were found.

Stratified seeds.- There were still significant differences in DTE and seedling weights among clones when the seeds were stratified. However, there were no significant correlations between DTE and any other measure of the stratified seeds.

Table 1. Comparisons among clones of means of seed properties. All mean weights are expressed in grams. Means followed by the same letter were not determined to be significantly different by Tukey's studentized range test (alpha = 0.05).

| CLONE | TOTAL SEED WEIGHT | SEED COAT WEIGHT | GAM/EMB WEIGHT | PERCENT OF SEED AS SEEDCOAT | PERCENT OF SEED AS GAM/EMB | LIPID WEIGHT | PERCENT GAM/EMB AS LIPID | CALORIES PER GRAM GAM/EMB | CALORIES PER GAM/EMB |
|---|---|---|---|---|---|---|---|---|---|
| H | 0.0390 a | 0.0266 a | 0.0124 abc | 68.0 a | 32.0 h | 0.0055 c | 40.0 ij | 6613 ghi | 90.7 ab |
| J | 0.0376 a | 0.0247 a | 0.0129 ab | 65.7 abc | 34.3 fgh | 0.0049 cde | 39.6 ij | 6605 ghi | 82.1 bcd |
| S | 0.0374 a | 0.0255 a | 0.0119 bcd | 68.1 a | 31.9 h | 0.0052 cd | 56.8 c | 6942 bc | 65.5 fg |
| N | 0.0317 b | 0.0180 bcde | 0.0137 a | 56.8 g | 43.2 b | 0.0050 cde | 46.9 edf | 6750 defg | 73.7 def |
| I | 0.0303 bc | 0.0185 bcd | 0.0119 bcd | 60.5 def | 39.5 cde | 0.0041 fg | 34.5 k | 6505 i | 77.6 de |
| E | 0.0301 bc | 0.0191 b | 0.0109 de | 63.4 bcd | 36.6 efg | 0.0042 efg | 47.4 def | 6759 defg | 60.8 gh |
| D | 0.0301 bc | 0.0185 bcd | 0.0116 bcd | 61.3 def | 38.7 cde | 0.0045 def | 57.9 c | 6964 bc | 55.4 h |
| G | 0.0298 c | 0.0179 bcde | 0.0119 bcd | 59.7 efg | 40.3 bcd | 0.0045 def | 40.9 hi | 6632 efghi | 72.8 ef |
| K | 0.0298 c | 0.0185 bcd | 0.0113 cd | 61.9 def | 38.1 cde | 0.0051 cd | 43.8 fghi | 6687 defgh | 79.1 de |
| F | 0.0297 c | 0.0160 ef | 0.0137 a | 53.5 h | 46.5 a | 0.0046 cde | 40.3 ij | 6619 fghi | 77.0 de |
| L | 0.0297 c | 0.0188 bc | 0.0110 de | 63.3 bcd | 36.7 efg | 0.0065 b | 50.8 d | 6825 cd | 87.9 abc |
| Q | 0.0267 d | 0.0153 fg | 0.0114 cd | 57.3 g | 42.7 b | 0.0047 cde | 48.1 def | 6772 def | 66.2 fg |
| O | 0.0266 d | 0.0168 cdef | 0.0098 ef | 63.2 bcd | 36.8 efg | 0.0035 gh | 44.9 efgh | 6710 defg | 52.9 h |
| P | 0.0255 de | 0.0160 ef | 0.0095 fg | 62.8 bcd | 37.2 def | 0.0066 b | 48.4 de | 6779 de | 92.6 a |
| B | 0.0242 ef | 0.0163 def | 0.0080 h | 67.3 a | 32.7 h | 0.0028 h | 36.1 jk | 6537 hi | 53.0 h |
| M | 0.0242 ef | 0.0162 def | 0.0079 h | 67.5 a | 32.5 h | 0.0078 a | 70.2 a | 7205 a | 81.2 cde |
| A | 0.0239 ef | 0.0157 ef | 0.0081 gh | 66.1 ab | 33.9 gh | 0.0052 cd | 45.7 efg | 6726 defg | 76.7 de |
| R | 0.0236 fg | 0.0149 fg | 0.0087 fg | 63.1 bcd | 36.9 efg | 0.0054 c | 63.3 b | 7070 ab | 61.5 gh |
| C | 0.0221 g | 0.0131 g | 0.0090 fg | 59.0 fg | 41.0 bc | 0.0050 cde | 41.7 ghi | 6647 efghi | 79.2 cde |
| MEANS | 0.0291 | 0.0182 | 0.0108 | 62.5 | 37.5 | 0.0050 | 47.2 | 6755 | 72.9 |

Table 2. Mean numbers of days to seedling establishment (DTE), establishment values (EV), mean seedling weights, and comparisons among clones. Standard deviations (SD) of the mean DTEs show how the variance was reduced by stratification. Seedlings from unstratified seeds and stratified seeds were grown for 97 and 75 days, respectively. Means followed by the same letter were not determined to be significantly different by Tukey's studentized range test (alpha = 0.05).

| | UNSTRATIFIED SEEDS | | | STRATIFIED SEEDS | | | SEEDLING WEIGHT (grams) | |
|---|---|---|---|---|---|---|---|---|
| CLONE | MEAN DTE | SD | EV | MEAN DTE | SD | EV | UNSTRATIFIED SEEDS | STRATIFIED SEEDS |
| Q | 55.4 a | 23.5 | 0.23 | 18.9 a | 12.3 | 9.94 | 0.1998 de | 0.3432 abc |
| K | 49.4 ab | 23.5 | 0.56 | 12.4 cd | 3.7 | 0.56 | 0.3161 cde | 0.2424 bc |
| N | 47.1 ab | 20.7 | 0.40 | 15.3 abc | 8.6 | 17.41 | 0.4157 abcde | 0.4387 a |
| S | 45.9 ab | 20.1 | 0.50 | 18.2 ab | 4.8 | 15.16 | 0.2265 de | 0.3288 abc |
| C | 41.9 abc | 17.2 | 0.39 | 13.5 bcd | 6.8 | 12.49 | 0.1439 e | 0.2087 bc |
| G | 38.6 bcd | 23.6 | 1.17 | 13.6 bcd | 8.6 | 23.87 | 0.4952 abcd | 0.3984 ab |
| H | 36.1 bcde | 21.8 | 0.78 | 12.6 cd | 3.3 | 22.38 | 0.3747 bcde | 0.3712 abc |
| J | 35.9 bcde | 14.2 | 0.56 | 10.6 cd | 3.1 | 26.85 | 0.3254 cde | 0.3868 abc |
| R | 34.3 bcdef | 21.2 | 0.67 | 13.0 cd | 3.9 | 14.72 | 0.4674 abcde | 0.3675 abc |
| F | 33.7 bcdef | 24.9 | 2.63 | 12.4 cd | 2.7 | 21.86 | 0.5216 abcd | 0.3996 ab |
| I | 29.0 cdefg | 16.7 | 2.17 | 10.7 cd | 2.0 | 24.98 | 0.4370 abcde | 0.3266 abc |
| A | 27.3 cdefg | 11.7 | 2.11 | 11.1 cd | 1.6 | 36.10 | 0.3800 abcde | 0.3757 abc |
| O | 22.9 defg | 15.9 | 3.67 | 11.3 cd | 2.2 | 28.88 | 0.6145 abc | 0.4253 a |
| D | 21.9 efg | 15.4 | 7.19 | 10.7 cd | 2.9 | 26.85 | 0.6472 abc | 0.4526 a |
| B | 21.5 efg | 8.2 | 1.41 | 12.7 cd | 6.0 | 6.42 | 0.3821 abcde | 0.2891 abc |
| L | 18.6 fg | 13.0 | 5.50 | 10.4 cd | 2.0 | 33.43 | 0.6482 abc | 0.4313 a |
| P | 17.6 g | 6.5 | 6.78 | 12.1 cd | 2.7 | 24.48 | 0.7042 ab | 0.4186 ab |
| M | 16.6 g | 16.6 | 2.28 | 11.9 cd | 2.5 | 24.47 | 0.5052 abcd | 0.3947 ab |
| E | 14.9 g | 3.9 | 14.01 | 10.8 cd | 3.9 | 23.00 | 0.7254 a | 0.3963 ab |
| MEANS | 31.1 | 20.9 | 2.80 | 12.6 | 6.3 | 21.03 | 0.4484 | 0.3691 |

Table 3. Summary of important regression models for the dependent variable seedling weight. Unless indicated, all parameter estimates for independent variables were significant at alpha = 0.05.

| MODEL | INDEPENDENT VARIABLES | R-SQUARE |
|---|---|---|
| For Unstratified Seeds | | |
| 1 | DTE | 0.63 |
| 2 | DTE, calories per gam/emb | 0.76 |
| 3 | DTE, gam/emb weight | 0.76 |
| For Stratified Seeds | | |
| 4 | coat weight, gam/emb weight, total weight | 0.37 |
| 5 | coat weight, gam/emb weight, total weight, calories per gam/emb | 0.50 |
| 6 | EV | 0.46 |
| 7 | EV, coat weight, gam/emb weight, total weight | 0.72 |

[1] parameter estimate significant at alpha = 0.10

Although other models had higher $R^2$ values, Model 4 ($R^2$ = 0.37) would be the easiest to use because it requires only weights which are easily obtained. Calories per gam/emb accounted for an additional 13% of the variation in seedling size (Model 5). However, the parameter estimate for calories was significant only at the 90% level. These were the only models in which DTE and seed properties accounted for a large amount of the variation in the weight of seedlings from stratified seeds.

All models were retested, substituting EV for DTE. Model 6, with EV as the only independent variable, accounted for 46% of the variation. The weights of the seed parts and total seed weight to the model accounted for another 26%.

## DISCUSSION

The total seed weight, often proposed as being related to seedling size, was of no predictive value in this study. The seed coat represented the major portion of the seed (approximately 63% by weight). However, seed coat weight was not closely correlated with the gam/emb weight (R = 0.51), but was highly correlated with the total seed weight (R = 0.95). These correlations demonstrate that seed coat weight may change among some clones without relative changes or even absolute changes in gam/emb weight. Some seeds were heavier only because they had heavier seed coats.

It might be expected that larger seed coats would offer greater mechanical restriction, have more inhibitory chemicals, etc., and result in longer germination and establishment times (Barnett 1972). However, there was no correlation between seed coat weight and DTE, which does not support this view. Gam/emb weight appears to be more important than seed coat weight in determining seedling size. Total seed weight, primarily determined by the seed coat weight, is a poor predictor of seedling weight.

The EV accounted for variation in the weights of seedlings from stratified seeds that the DTE could not explain. The establishment (germination) value varies directly and proportionally with both speed of establishment and total establishment and is relatively sensitive to minor differences in either. The EV is an absolute value while the DTE is a mean. Since there were few differences between DTEs or seedling weights for stratified seeds, it was not likely that a relationship between the two could be developed. In contrast, the EVs spanned a large range and the differences among clones in them were quite large. For unstratified seed, there were many differences in DTE and it did not matter if DTE or EV was used to develop relationships.

## CONCLUSIONS AND IMPLICATIONS

As expected, there were large differences among clones in all of the seed and seedling properties examined. There were no simple relationships between total seed weight and seedling performance. Seed size should be closely correlated with seed weight. It does not appear that sorting seeds by size is a useful nursery practice for controlling the size of seedlings. Rather, seeds should be planted separately by clone, a standard practice in some nurseries.

Many attempts to reduce the time required to test the performance of tree progenies have been unsuccessful. Seed size has often been thought to be related to early progeny performance, and has been proposed as a possible quick indicator of progeny performance. But the correlation between seed size and progeny performance is usually poor.

In attempts to shorten progeny testing, age:age correlations (i.e., juvenile:mature correlations) are used to determine if differences among clones in progeny characteristics (i.e., height) are consistent throughout the lives of the plants. The correlations between seedling size and size at older ages decrease with time, but do not disappear. Despite some arguments to the contrary, trees that start out big seem to remain big (Bengston 1963, Grigsby 1975, Hatchell et al. 1972, Sluder 1979, Wakely 1969, Zarger 1965). Indeed, exponential growth models demonstrate that the rate of growth is a function of the initial size of the organism (Lotka 1956). The effect of initial size can be amplified in the nursery where large seedlings often suppress smaller ones.

If half to three-quarters of the variation in seedling size is attributable to nongenetic and nonheritable properties of the seeds, and if seedling size is correlated to tree size, then estimates of genetic gain may be overstated. When genetic gain is calculated from progeny tests, the effect of initial size is not considered. Thus, it becomes included in the estimates of genetic gain. Along with heritable factors, there are many nonhereditary factors and cultural practices that affect seed properties. Energy content and the number of days to seedling establishment (DTE) may also be strongly influenced by these factors.

The small embryo represents less than 10% of the total seed and is the only part that contains chromosomes from both parents. Maternal influences on the seed may have many effects on the resulting seedling (Perry 1976). First generation progeny tests may partly select the best seeds rather than genetically superior progeny. While there is certainly genetic gain as a result of progeny testing, it is likely that it is not being accurately estimated. To accurately predict future growth on the basis of early progeny performance, other genetic and nongenetic factors that regulate germination and growth must be taken into account.

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