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EFFECT OF GENETICS ON SURVIVAL OF LOBLOLLY PINE SEEDLINGS

by
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INTRODUCTION

Outplanting survival is related to genetics. For example, slash pine is not planted in Canada and certain Coastal Plain loblolly pine families (e.g. 7-56) are not planted on the Cumberland Plateau. In some artificial freezing trials, survival of unimproved piedmont sources (after exposure to -15°C) may be 21 to 32 percentage points higher than survival of improved sources (Kegley 1999). For loblolly pine in field trials, the heritability for survival might range from 0.49 to 0.89 (Table 1). Of course the heritability will be zero when all families have 100% survival. This may occur when the “planting chance”[1] has excellent environmental conditions for survival (e.g. good soil moisture, optimal temperatures, low wind, adequate rainfall, careful planting methods).

In 2007, a regeneration forester expressed concern about low outplanting survival on 5% of their planting chances (i.e. survival ranged from 40% to 59%). Average initial survival for 158 sites was 74% and low survival on 8 family block plantings appeared to be related to a hard freeze. Some foresters commented that when planting orchard-mix lots, seedling survival below 60% was rare. Although some geneticists report an increase in survival with family-block planting (Gladstone et al. 1987), others questioned if block-planting might partly explain low survival when conditions for survival are not optimal.

Three approaches were used to examine the effects of genetics on early survival. The first approach involved an examination of early survival data from 30 unimproved genotypes (Beineke 1966). This dataset was collected since it demonstrates that genetics, planting quality and seedling quality can affect initial survival. The second approach involved an examination of the range in survival observed from six-year-old progeny tests. This dataset was used to illustrate how the range in observed survival increases as the average survival for the test approaches 50%. Finally, a computer simulation was conducted to illustrate why the range in survival among planting chances increases when an organization switches from planting mixed-lots to planting in pure family-blocks.

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[1]The term “planting chance” will be used in this publication since the term, in some cases, is more accurate than “planting site.” In some reports, planting failure is not due to the “site”, per se, but instead is a function of the “planting chance.” Some planting chances are more successful than others due to genetics involved or the environmental conditions associated with the planting chance. Many foresters will agree that the planting crew can also make a difference in survival.

METHODOLOGY

Bareroot loblolly pine: Seed were collected from 30 "wild" mother trees near Raleigh, NC and in 1963, seed were sown in a bareroot nursery at Clayton, NC. A planting date study was established at the Schenck Forest near Raleigh. Seedlings were harvested approximately one week before planting. Planting dates were December (15-18), January 27, March 10, April 21 and June 2. The field design consisted of five randomized blocks with one row of 20 seedlings in each block (i.e. 100 seedlings planted per family-date combination). The entire study consisted of 15,000 seedlings. New root growth (> 6 mm) of seedlings in the field was determined after 90 days (December planting), 60 days (January planting), and 30 days (March, April, June plantings). Details regarding the study were provided by Beineke (1966).

Container-grown loblolly pine: Dr. Bailian Li with the NCSU Tree Improvement Cooperative provided survival data from numerous (2nd cycle) progeny trials (often grown in Ray-Leach containers). Trials included various families with 6-tree row tests (replicated 6 times) from which, five trials were selected to illustrate the range in survival by family.

Statistical tests: To detect differences in survival for the bareroot study, an ANOVA was conducted using replication and family in the model (Beineke 1966). A contrast test was conducted to compare the survival of family A9 or B12 with the survival of the 29 remaining families. In addition, a different method of means separation was conducted. The 5% outlier method involves using an Exact Method (Blyth and Still 1983) to calculate low and high confidence intervals (with a 90% confidence interval). An online program (http://www.measuringusability.com/wald.htm#exact) was used to calculate the low and high intervals. This method assumes the reported mean survival for a site is equal to the "true mean" for the planting chance. To calculate this confidence interval, one only needs to know; (1) the overall "planting chance" mean survival, and (2) the number of seedlings used to calculate a family mean (e.g. n = 100 for the 1963 study; n = 36 for the NCSU Tree Improvement Coop trials). Families that lie outside the confidence interval are flagged as outliers.

Computer simulation: A computer simulation was conducted to examine the potential effect of outplanting seedlings in family blocks instead of in a seed orchard mix. Ten families were examined assuming the "true survival" of the families were; 95%, 90%, 85%, 82%, 80%, 78%, 75% 74%, 65%, and 57%. This hypothetical example results in a range in survival of 40 percentage points. For each planting chance, survival for individual seedlings was determined at random. There were 1,000 seedlings for each family/planting chance mean. In total, there were 150 planting chances for each family and 150 planting chances for each mixed-lot. The mix-lot contained 100 seedlings from each of the ten families. A histogram was used to compare the results from each method of genetic deployment.

RESULTS

BAREROOT

December planting chance: Frozen ground occurred in December when air temperature (Raleigh-Durham Airport) dropped to 18, 15, 11, and 22 F on Dec 15th, 16th, 17th and 18th, respectively (Figure 1). Nearly all roots were frozen prior to planting. On the coldest day of the month (Dec 21st), the temperature reached 8 F. Apparently, there was a great difference among planting crews, particularly in depth of planting. The variation among blocks was, in part, due to frost heaving caused by shallow planting in some blocks. Block #5 had excellent initial survival with 93.3% seedlings living after 100 days (i.e. only 10 seedlings died). In contrast, block #1 had the greatest mortality with 53.3% survival (Figure 2).

January-March-April planting chances: Average survival was greater than 97% for the January, March and April planting dates and soil moisture was "high" for all three dates. Planting rates improved over time with 3,000 seedlings planted in 7 hours (Jan), 6 hours (March), 4.5 hours (April), and 3 hours in June.

June planting chance: Seedling quality was low when actively growing seedlings were lifted in late May. At this time, the nursery soil was dry and there was some root damage during lifting. Seedlings from this lifting date were about twice as tall as seedlings lifted in January and therefore were more "out-of-balance." They also had lower root-growth potential than seedlings lifted before May. As a result, average initial survival was 57.5% and the range in observed survival was the greatest (Figure 3).

CONTAINER

Survival for the genetic trials (Figure 4) was recorded six years after planting. Therefore, survival rates for the first year are unknown. A sample of five diallel tests were selected (Figure 5) and confidence limits were generated to detect the number of families with below the "expected" survival range (Table 3).

COMPUTER SIMULATION

The ten families selected for a computer simulation had survival means similar to ten families in a "real world" progeny test (148-4; see Table 3). However, unlike the real world, the 150 planting chances (i.e. 15 per family) had the exact same environmental conditions and the only source of variation was due to random chance. For example, for the family with 80% survival, the range in survival varied from a low of 79% to a high of 83%. For mixed lots (containing all 15 families, the range varied from a low of 76% to a high of 82%. This simulation illustrates why the range in observed survival increases when one deploys families in pure blocks (Figure 6). This wider range in survival is supported by data from progeny tests (e.g. when the standard deviation is 10% or more, compare the range in survival among blocks with the range in survival among families).

DISCUSSION

In the North Carolina study, initial survival (60 to 100 days after planting) was high except when planting in frozen soil (i.e. December) or when planting unbalanced, actively-growing stock prior to the hot summer (June). According to contrast tests (using arcsine square-root transformed percentages), family A9 had lower survival when planted in January, April and June (Table 2) while family B12 had low survival in January and March. Family A-9 family was tallest in the nursery, had few lateral roots, transpired more per day than other genotypes and had a low root-growth (Beineke 1966).

There are four basic approaches to the operational use of A-9 type families. Some will outplant A-9 in blocks and hope that good environmental conditions will favor good survival. Others will attempt to improve survival by culturing A-9 differently in the nursery (perhaps with top-pruning or growing this family in containers). Some will use A-9 as part of a mixed-seedlot in hopes that other families will increase the average survival for the planting chance. The fourth option (which is rarely taken) is to remove this low-survival family from the seed orchard.

Seed orchard mix vs. family blocks
The computer simulation demonstrates that the range in seedling survival noticed by regeneration foresters will increase when an organization switches from deploying seedlings from an orchard mix to deploying half-sib or full sib families. When using an orchard mix, there were no planting chances with less than 75% survival. In contrast, when utilizing family block planting, 20% of the planting chances fell below 75% survival. Removing the poorest surviving family would increase average survival of the orchard mix by about 2.5 percentage points (data not shown). Likewise, removing the poorest surviving family from deployment as a pure family block would cut in half the number of planting chances with less than 75% survival (data not shown).

When we plant either pure clones or a full-sib family, the genetic base declines and we start to see more differences in seedling characteristics that are due to genetics. This applies not only to factors such as branch angle and bark thickness, but it also applies to survival on an operational level. Survival obtained in a research study is often higher than survival obtained by operational hand planters; and the high "research" survival can mask the genetic effect. However, when research plantings are established at the edge of the natural range, environmental extremes can cause problems in survival (Wells and Lambeth 1983). Recently, one full-sib family of loblolly pine (L5) died suddenly at age 8 years near Sanderson, Florida (see Staudhammer et al. 2009 for study details).

5% confidence limit
The 5% confidence limit method (for detecting low-survival outliers) is applicable for use on survival percentages that have a binomial distribution (e.g. January and March planting chance). In some cases, it will be more conservative than a test Duncan's test that is conducted with a non-significant F-test (see Table 2). For example, the 5% limit did not detect any outliers for the January or March planting dates but the Duncan's Multiple Range Test detected differences among certain genotypes. A disadvantage of the "5% method" is that does not determine if an outlier exists because of genetics or some other factor.

MANAGEMENT IMPLICATIONS

When seedling survival routinely is greater than 75% in the field, there may be little concern over the effect of genetics on outplanting survival (Lambeth 2000). However, when outplanting survival is less than 60%, foresters will sometimes first look to the nursery for an explanation of low survival. There are many examples where the nursery was not to blame. In some cases, low survival was due to shallow planting by researchers (e.g. replication #1 in the December planting chance) or by poor hand planting by operational crews (Rowan 1987; South and Mitchell 1999).

In a few cases, low survival may be attributable to deploying half-sib or full-sib families in blocks. Low survival for some families (e.g. A9) may be due to genetics that produce few lateral roots and a low root-weight ratio. Field foresters should realize that genetics has an effect on both early survival (Beineke 1966, Kegley 1999) as well as survival at age 25 years (Wells and Lambeth 1983). Therefore, deploying families in blocks (as opposed to mixed-lots) will naturally increase the range in survival among planting chances.

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Table 1. Average survival (age 6 years) and family mean heritability for a total of 44 separate diallels (NCSU 1995; p 13).

Region	**Average Survival (%)**	**Heritability**
Coastal Virginia	95	0.84
Southern MS and AL	93	0.76
Coastal GA and N. Florida	88	0.89
Northern MS and AL	87	0.49
Coastal South Carolina	82	0.58
Piedmont GA and SC	81	0.76

Table 2. The effect of lifting date on seedling morphology, root growth and initial survival.

	----- Planting chance -----				
	Dec. 15-18	January 27	March 10	April 21	June 2
Estimated lifting	Dec 8th	Jan 20	March 3	April 14	May 25
Shoot height	10.4	10.0	9.8	11.6	18.0
Root-weight ratio	0.23	0.21	0.23	0.26	0.19
Root growth (#)	1.4	7.1	4.9	69.1	16.5
Initial survival (%)	77.7	99.6	99.6	97.6	57.5
Family (P>F value)	0.7639	0.0004	0.0582	0.2126	0.0091
Range in initial survival	61-97	96-100	98-100	92-100	39-84
Standard deviation (%)	26.2	1.6	1.4	4.8	26.1
Survival LSD (5%)	28.4	1.7	1.7	5.1	25.8
A9 survival (%)	65	97	99	92	41
B12 survival (%)	73	96	98	98	46
A9 vs others (P > F)	0.2791	0.0002	0.2293	0.0088	0.0285
B12 vs others (P > F)	0.4683	0.0001	0.0027	0.8255	0.2385
90% confidence interval (%)	70-85	95-100	95-100	94-100	48-65
Outlier families with low initial survival (<5% confidence interval)	B6-68% C3-66% A12-66% A9 -65% A13-62% C11-61%	None	None	A9-93%	B12-46% C5 – 43% A9 – 41% C6 – 40% B6 – 39%
Family separation of transformed data using Duncan's Multiple Range test	*C11 less than top family (B8)	B12, A9 C6 less than top 24 families	A12, B7 and B12 less than top 23 families	*A9 and C8 less than top 5 families	A9, C6 B6 C5 less than top 3 families

(* unprotetected – F-test for family not significantly different α =0.06)

Table 3. Five examples (i.e. diallel tests) that illustrate the general effect of planting chance survival on the range of observed survival (age 6 year) by family (container stock).

	----- Diallel test -----				
	380-3	346-4	148-4	229-4	341-1
Mean survival (%)	62.5	72.0	82.9	92.9	99.1
# of families	27	29	27	29	30
# trees per family	36	36	36	36	36
Standard deviation (%)	14	19	10	5	2
Range in survival	36-86	39-100	60-97	80-100	91.7-100
90% confidence interval (%)	49-77	57-84	70-92	84-99	94-100
Number of outlier families with low survival	7	6	3	3	1

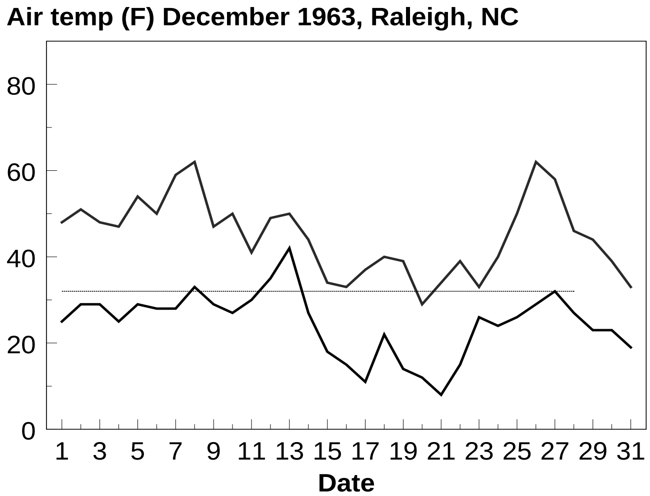

Figure 1. Maximum and minimum temperatures at the Raleigh Durham Airport, December, 1963.

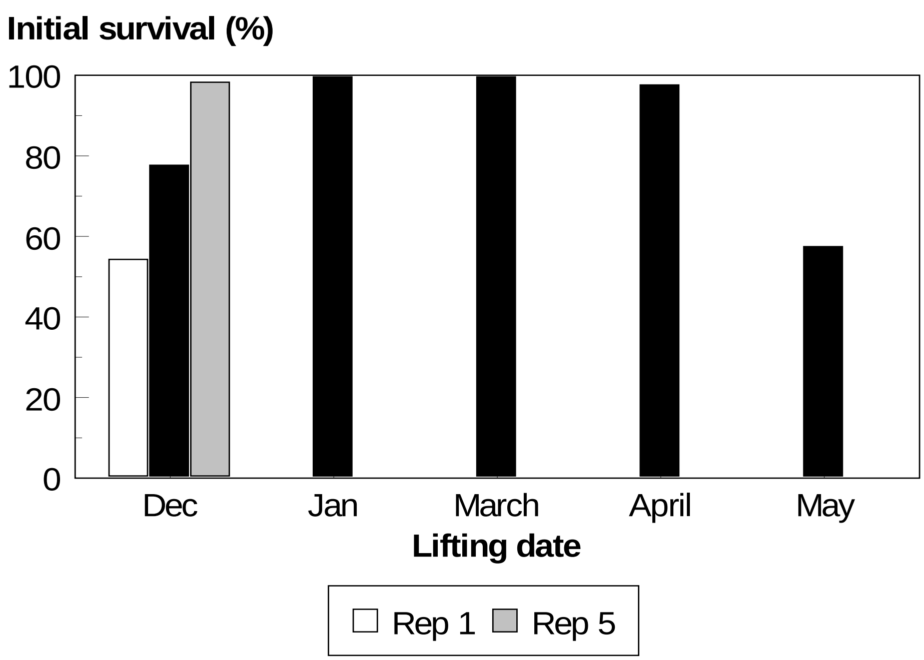

Figure 2. Effect of lifting date on average survival (black bars) of loblolly pine seedlings planted in the 1963-64 lifting season. Seedling survival when planting in frozen ground depends, in part on planting depth. For the first planting chance in December, planting quality in Rep 1 (white bar) was much lower than in Rep 5 (gray bar).

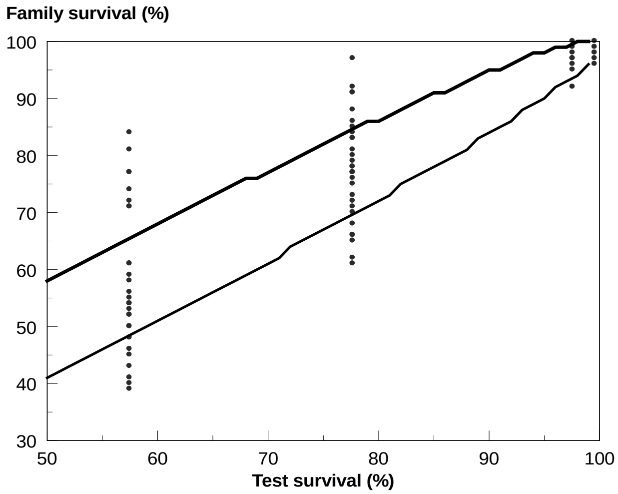

Figure 3. Effect of planting chance survival (x-axis) on range of family survival for bareroot loblolly pine (planted near Raleigh, NC). Each dot represents the survival of 100 trees from one family (i.e. mother tree). Some dots are hidden. The lines represent the 95% and 5% confidence limits assuming variation in family survival is due only to chance. Values below the lower line might be due to factors other than chance ($\alpha = 0.05$).

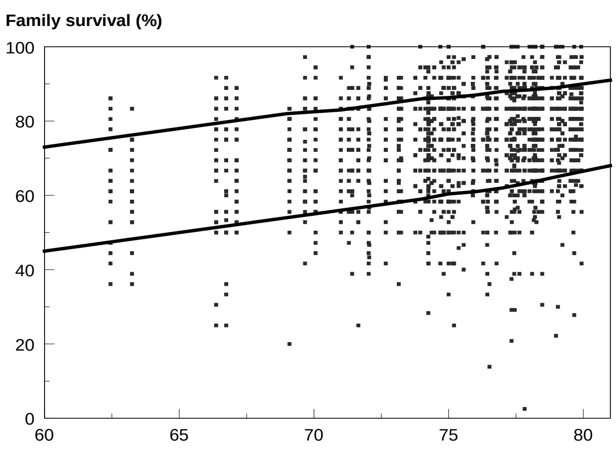

Figure 4. Effect of planting chance survival (x-axis) on range of survival of families (e.g. 29) for container-grown stock of loblolly pine. Each dot represents the survival (age 6 years) of 36 trees from one family. In this graph, there are 80 trials with 2282 dots (some dots are hidden). The lines represent the 95% and 5% confidence limits assuming variation in family survival is due only to chance. Data for trials with greater than 80% survival are not shown.

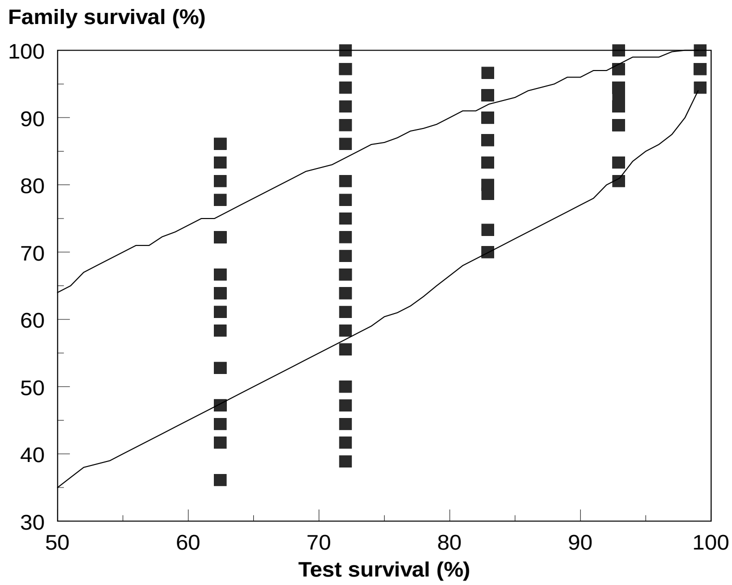

Figure 5. Effect of planting chance survival (x-axis) on range of family survival at age 6 years. Each dot represents the survival of 36 trees from one family. Some dots are hidden. The lines represent the 95% and 5% confidence limits assuming variation in family survival is due only to chance.

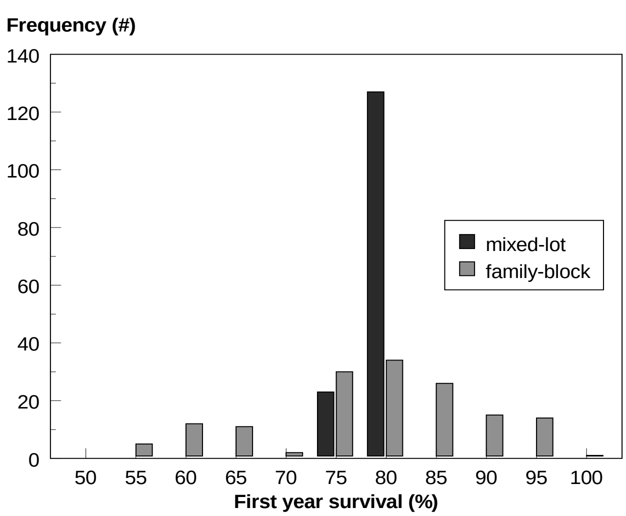

Figure 6. Effect of genetic deployment on expected survival of seedlings in a "virtual world." This histogram compares 150 planting chances that used an orchard mix (black bars) with 150 planting chances with family block planting. The range in observed survival is greater when the ten pine genotypes were deployed in family blocks (55-98%) rather than in an orchard mix (77-82%).